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(54) **HIV PRE-IMMUNIZATION AND IMMUNOTHERAPY**

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(57) **ABSTRACT**

The present invention relates generally to immunization and immunotherapy for the treatment or prevention of HIV. In particular, the methods include in vivo and/or ex vivo enrichment of HIV-specific CD4+ T cells.

17 Claims, 40 Drawing Sheets

Specification includes a Sequence Listing.

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(56)

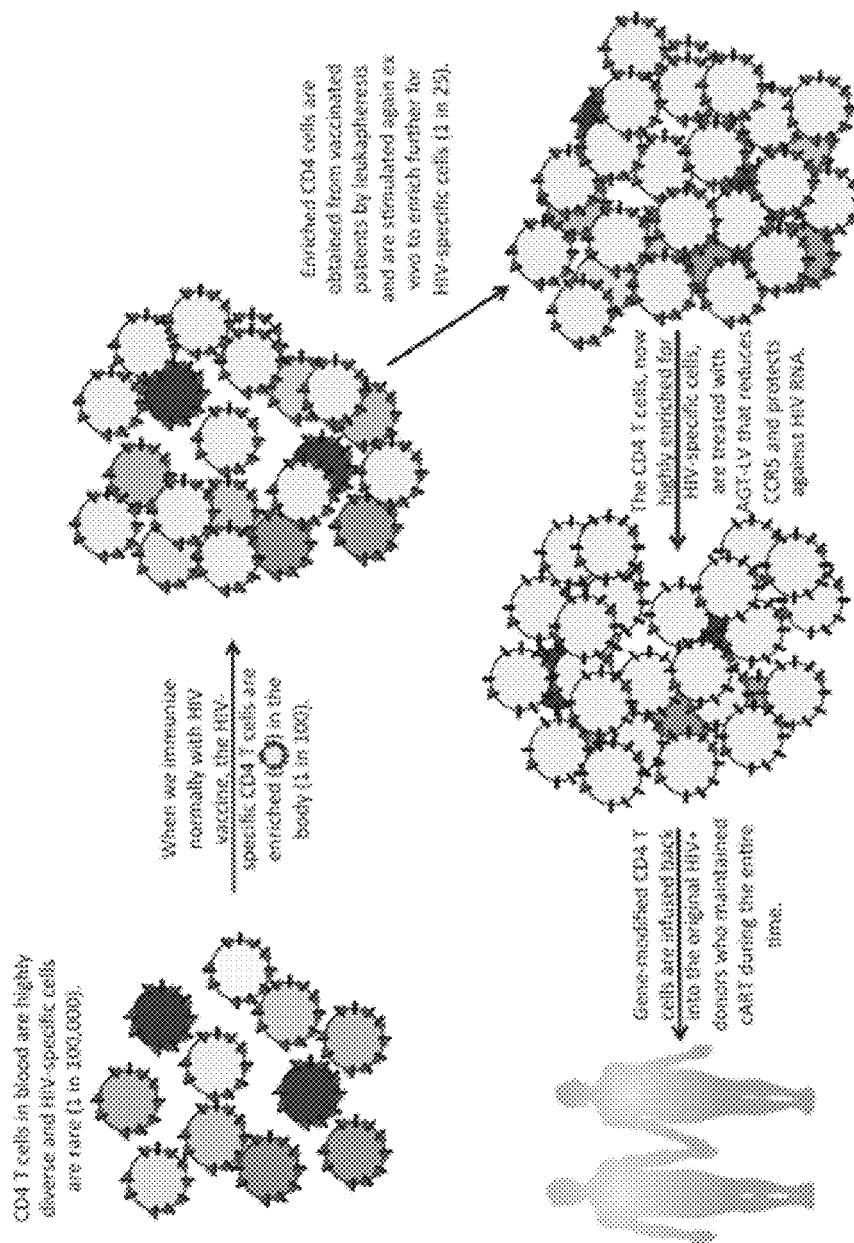
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 USPTO; Requirement for Restriction dated Aug. 3, 2018 in U.S. Appl. No. 16/011,550.
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 USPTO; Non-Final Office Action dated Dec. 31, 2018 in U.S. Appl. No. 16/182,443.
 EPO; Extended Search Report dated Dec. 12, 2018 in EP Application No. 16808223.8.
 EPO; Extended Search Report dated Dec. 11, 2018 in EP Application No. 16822021.8.
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 USPTO; Advisory Action dated Jul. 23, 2019 in the U.S. Appl. No. 15/736,284.
 USPTO; Notice of Allowance dated Aug. 14, 2019 in the U.S. Appl. No. 16/008,991.
 USPTO; Notice of Allowance dated Jul. 19, 2019 in the U.S. Appl. No. 16/132,247.
 EPO; European Search Report dated Aug. 12, 2019 in the EP Application No. 17764128.9.
 EPO; Supplementary European Search Report dated Sep. 6, 2019 in the Application No. 17750547.6.

* cited by examiner

Enrich (vaccine) and protect (AGT-LV) the HIV-specific CD4 T cells.

**FIG. 1**

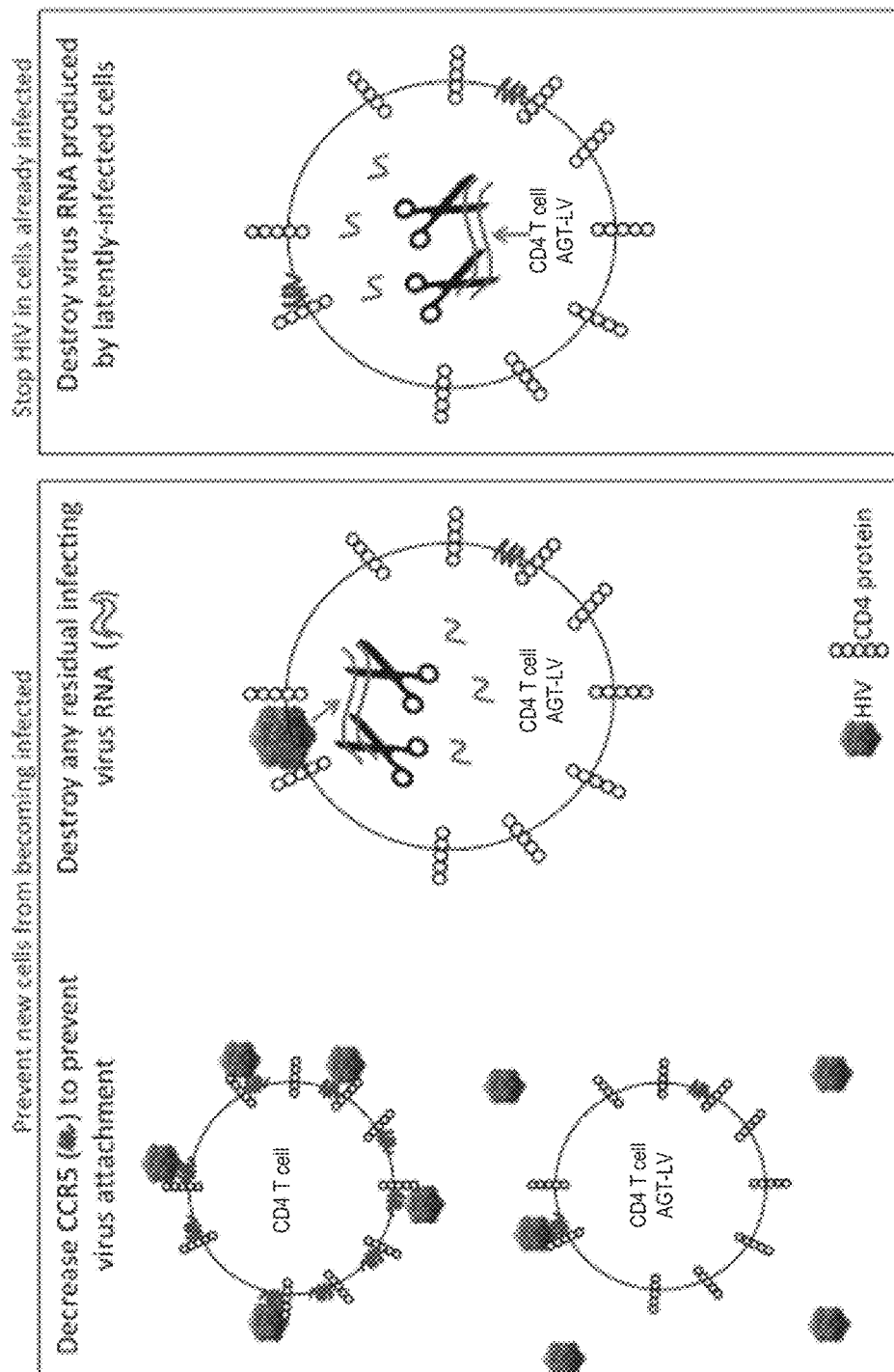


FIG. 2

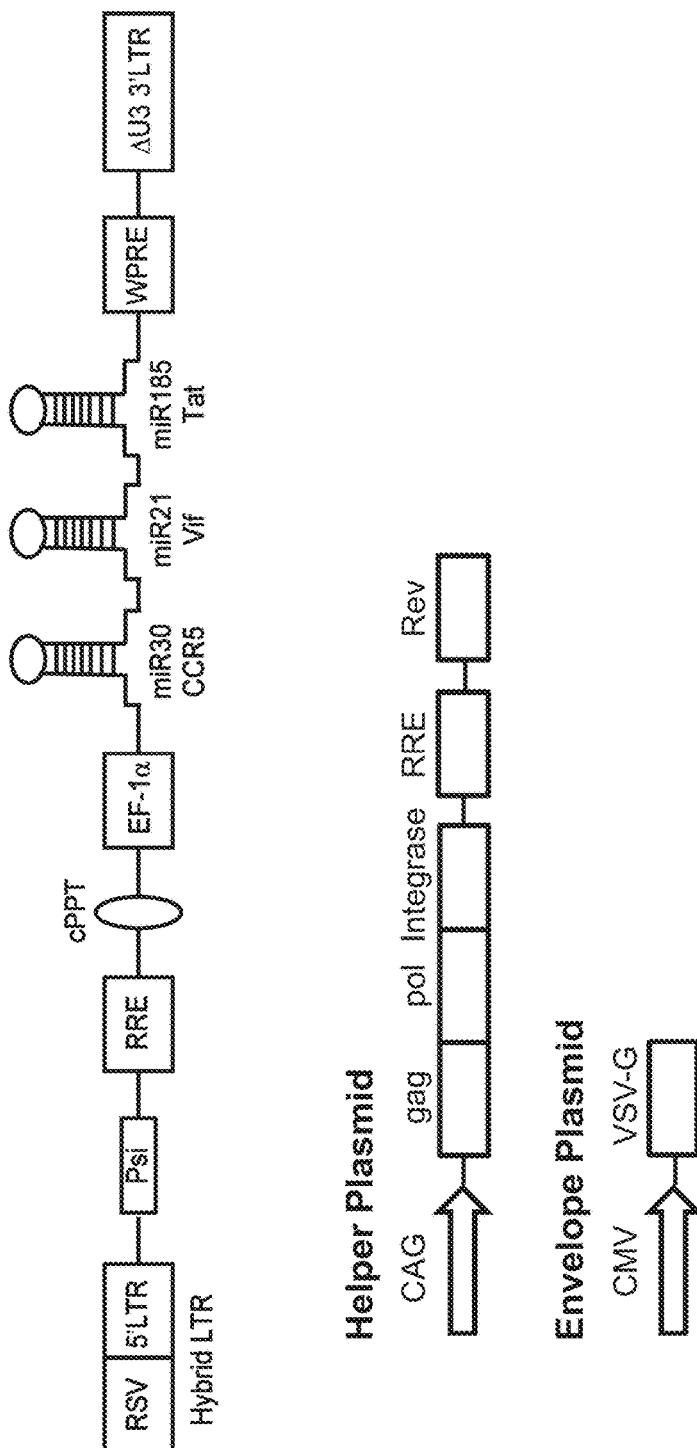


FIG. 3

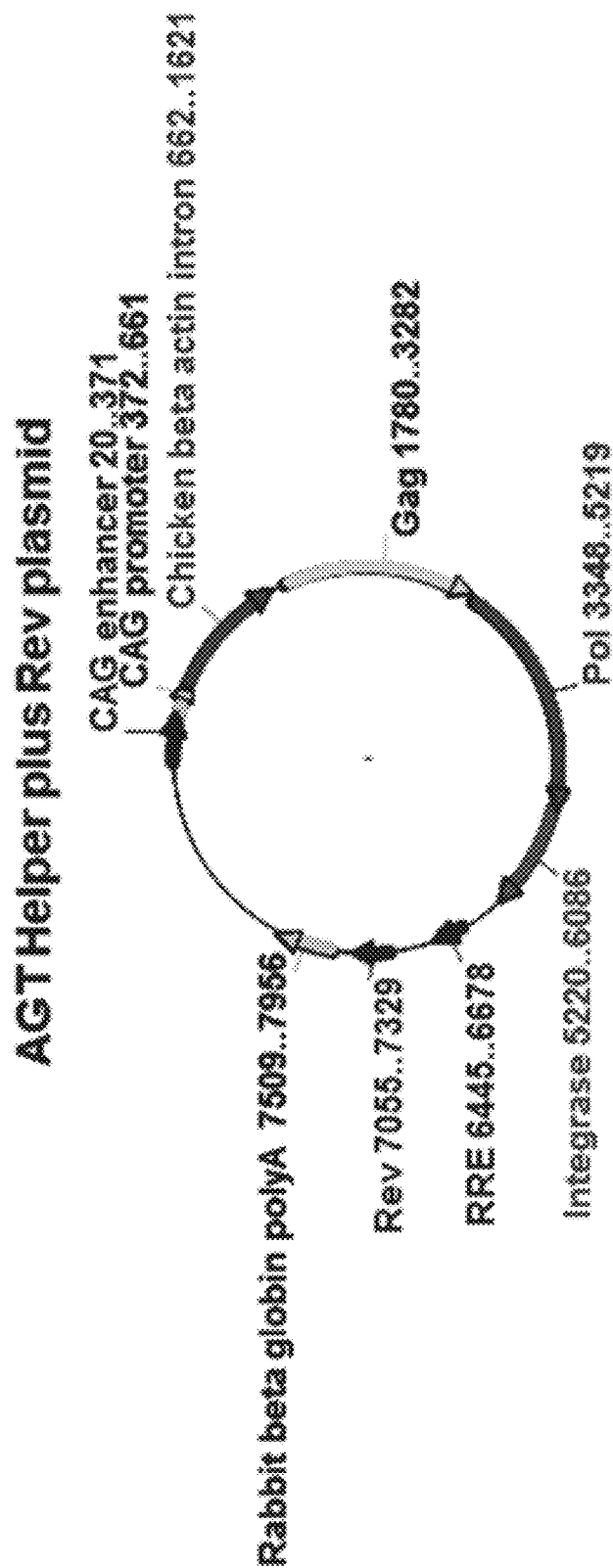


FIG. 4A

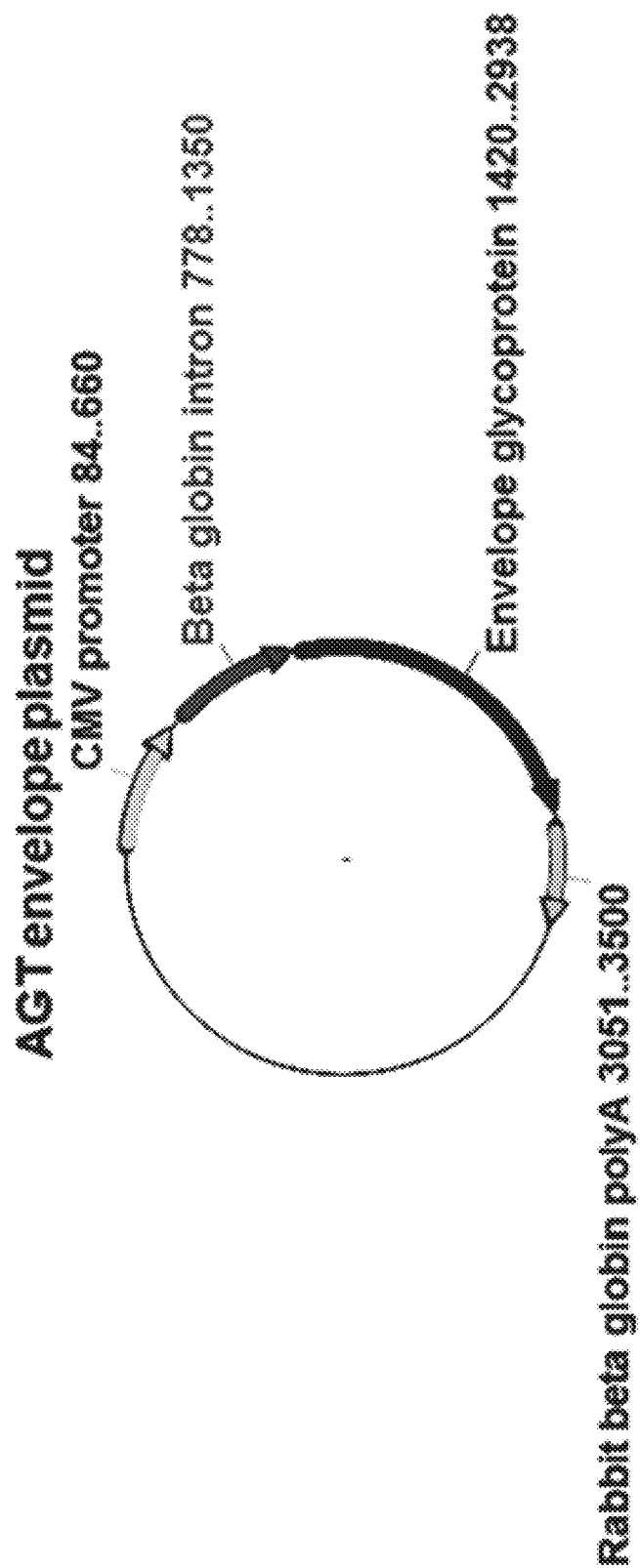


FIG. 4B

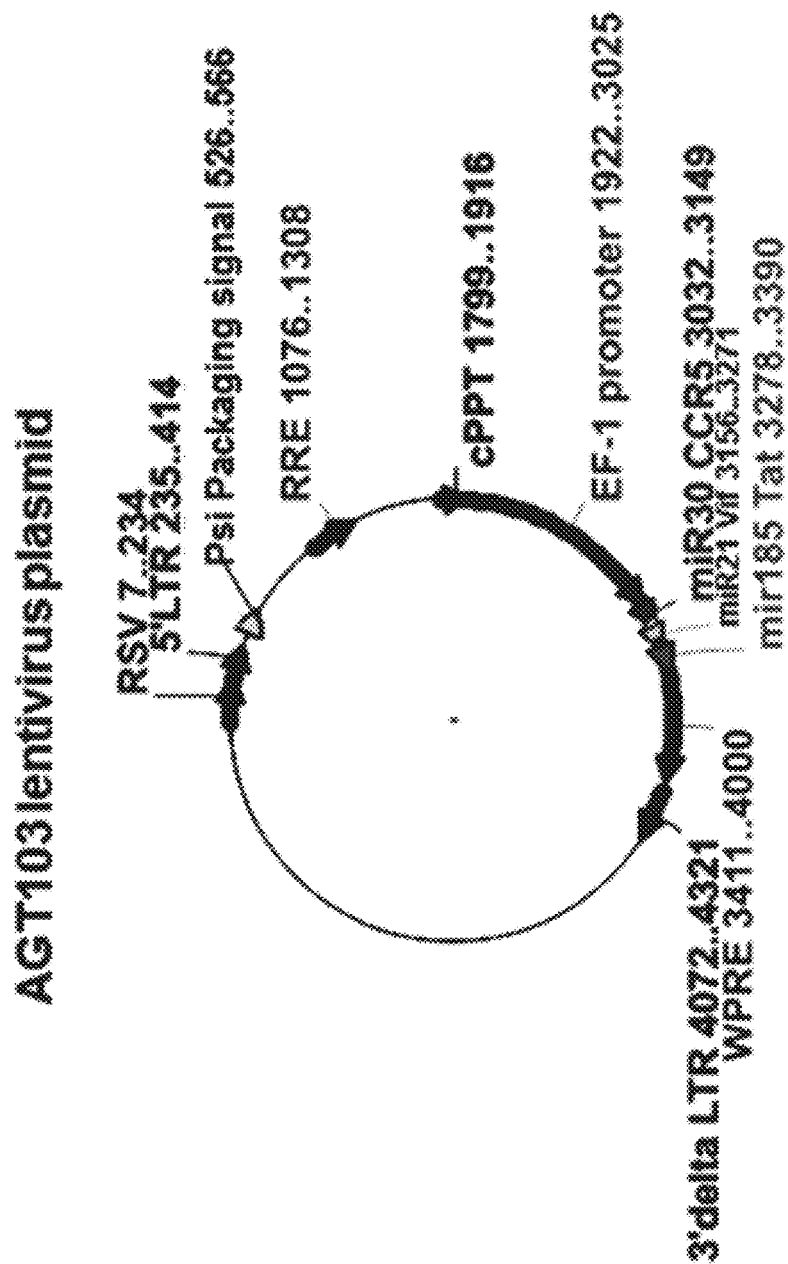


FIG. 4C

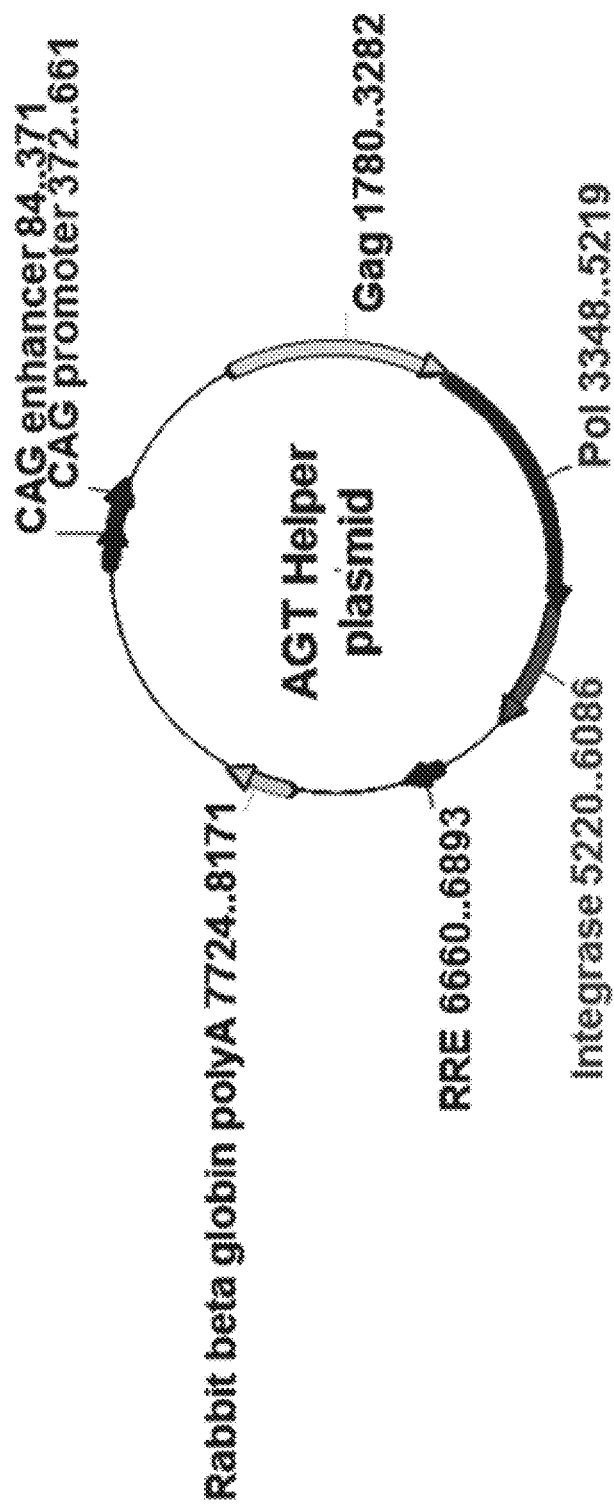


FIG. 5A

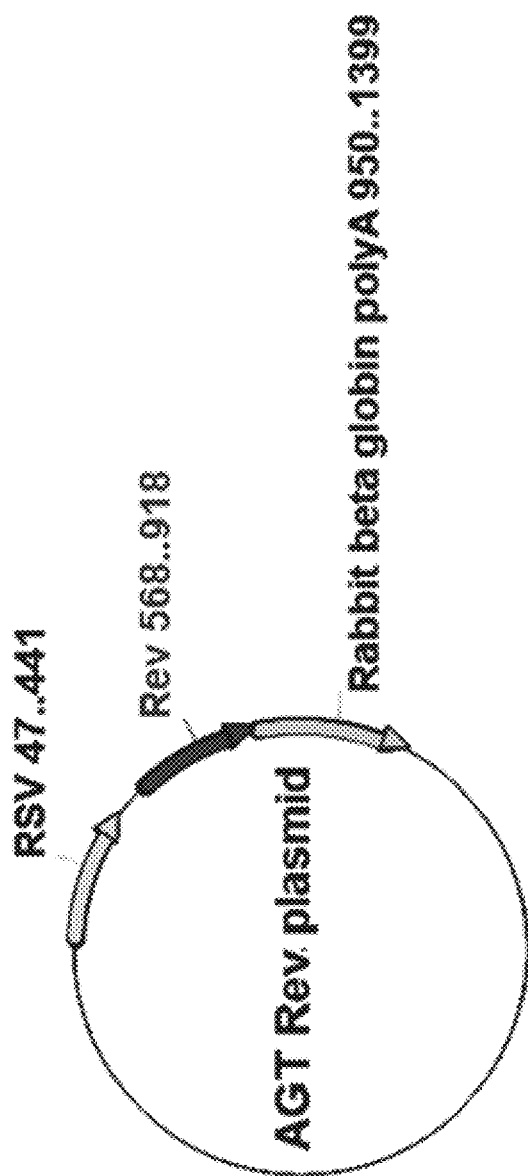


FIG. 5B

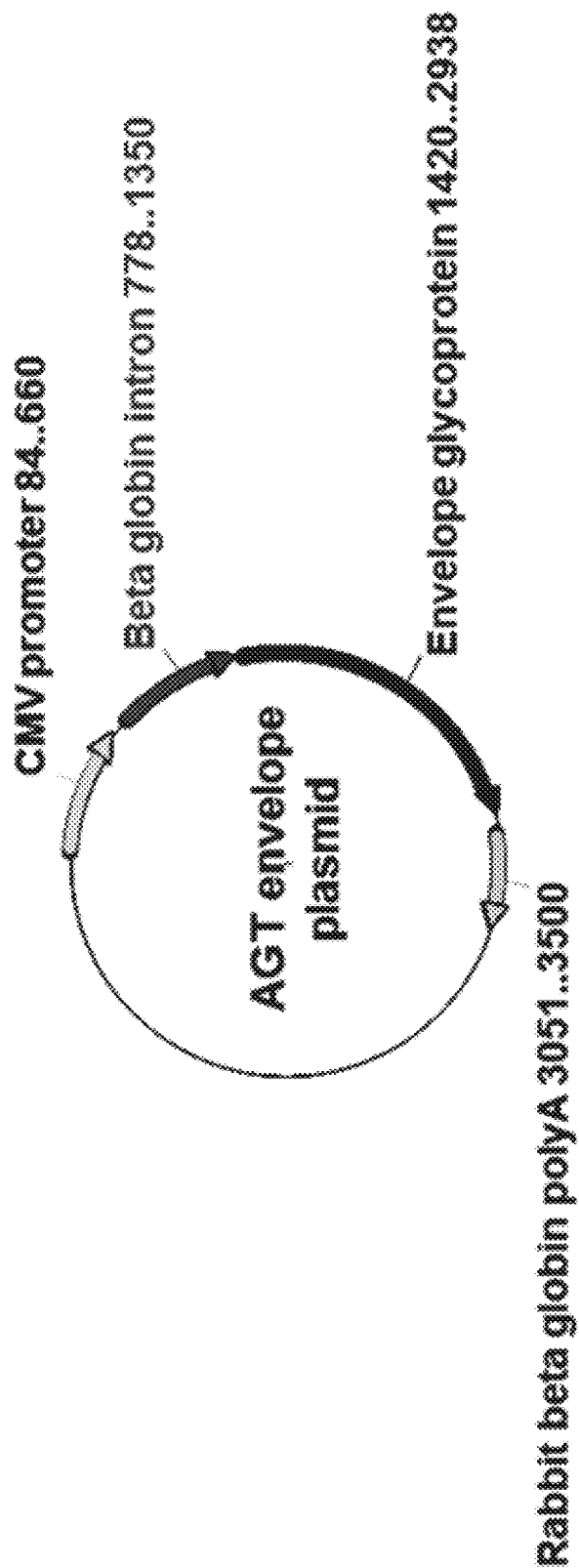


FIG. 5C

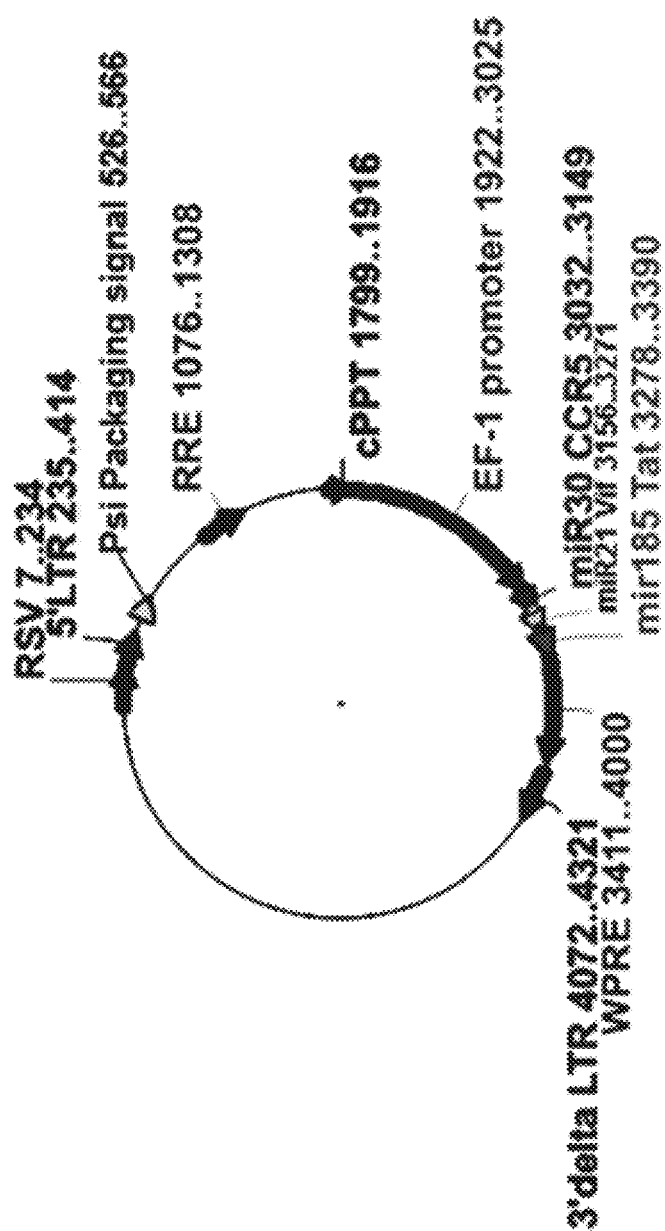


FIG. 5D

Elongation Factor-1 alpha (EF1-alpha) promoter

CCGGTGCCTAGAGAAGGTGGCGCGGGGTAAACTGGGAAAGTGATGTCGTGTACTGGCTCCGCCTT
TTTCCCCAGGGGTGGGGGAGAACCGTATATAAGTGCAGTAGTCGCCGTGAACGTTCTTTTCGCAA
CGGGTTTGCCGCCAGAACACAGGTAAGTGCCGTGTGTGTTCCCGCGGGCCTGGCCTCTTTACGG
GTTATGGCCCTTGCGTGCCTTGAATTACTTCCACGCCCCCTGGCTGCAGTACGTGATTCTTGATCC
CGAGCTTCGGGTTGGAAGTGGGTGGGAGAGTTCGAGGCCCTTGCCTTAAGGAGCCCCCTTCGCCTC
GTGCTTGAGTTGAGGCCTGGCCTGGGCGCTGGGGCCGCCCGCTGCGAATCTGGTGGCACCTTCGC
GCCTGTCTCGCTGCTTTTCGATAAGTCTCTAGCCATTTAAAATTTTGTGATGACCTGCTGCGACGCT
TTTTTTCTGGCAAGATAGTCTTGTAATGCGGGCCAAAGATCTGCACACTGGTATTTTCGGTTTTTG
GGGCGCGGGCGGGCGACGGGGCCCCGTGCGTCCCAGCGCACATGTTCCGGCGAGGCGGGGCTGCGA
GCGCGGCCACCGAGAATCGGACGGGGGTAGTCTCAAGCTGGCCGGCCTGCTCTGGTGCCTGGCCT
CGCGCCGCCGTGTATCGCCCCGCCCTGGGCGGCAAGGCTGGCCCGGTGGCACACAGTTGCGTGAG
CGGAAAGATGGCCGCTTCCCGGCCCTGCTGCAGGGAGCTCAAAATGGAGGACGCGGCGCTCGGGA
GAGCGGGCGGGTGAGTCACCCACACAAAGGAAAAGGGCCTTTCCGTCTTCAGCCGTCGCTTCATG
TGACTCCACGGAGTACCGGGCGCCGTCCAGGCACCTCGATTAGTTCTCGAGCTTTTGGAGTACGT
CGTCTTTAGGTTGGGGGGAGGGGTTTTATGCGATGGAGTTTCCCCACACTGAGTGGGTGGAGACT
GAAGTTAGGCCAGCTTGGCACTTGATGTAATTCCTTGGAAATTTGCCCTTTTGGAGTTGGATC
TTGGTTCATTCTCAAGCCTCAGACAGTGGTTCAAAGTTTTTTTCTTCCATTTCAGGTGTCGTGAT
GTACA

miR30 CCR5

AGGTATATTGCTGTTGACAGTGAGCGACTGTAAACTGAGCTTGCTCTACTGTGAAGCCACAGATG
GGTAGAGCAAGCACAGTTTACCGCTGCCTACTGCCTCGGACTTCAAGGGGCTT

miR21 Vif

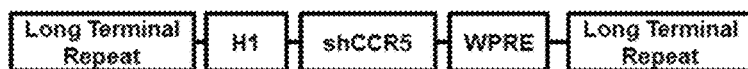
CCCCGGCATCTCCATGGCTGTACCACCTTGTCGGGGGATGTGTACTTCTGAACTTGTTGTTGAATC
TCATGGAGTTTCAAGAACAACATCCGCACTGACATTTTGGTATCTTTCATCTGACCA

miR185 Tat

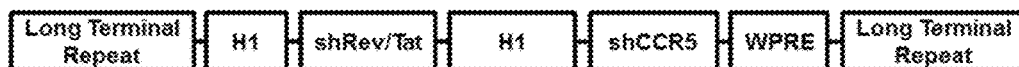
GCTAGCGGGCCTGGCTCGAGCAGGGGGCGAGGGATTCCGCTTCTTCTGCCATAGCGTGGTCCCC
TCCCCTATGGCAGGCAGAAGCGGCACCTTCCCTCCCAATGACCGCGTCTTCGTC

FIG. 6

Vector 1



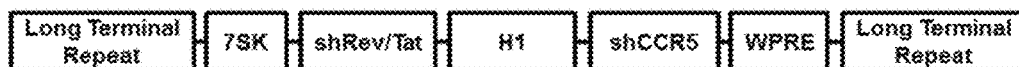
Vector 2



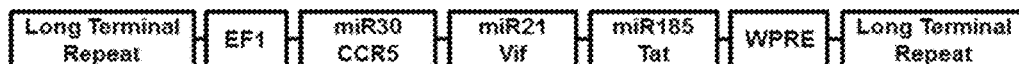
Vector 3



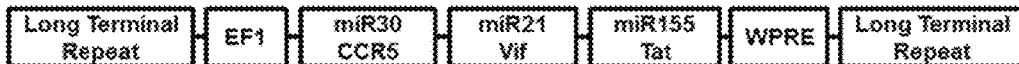
Vector 4



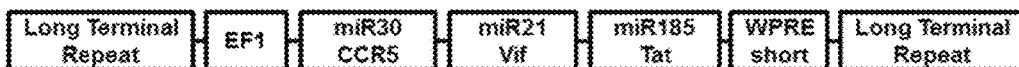
Vector 5



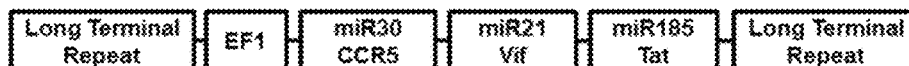
Vector 6



Vector 7



Vector 8



Vector 9

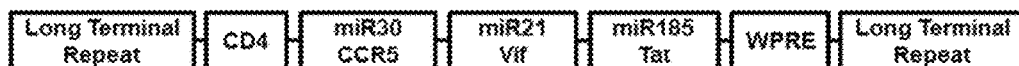


FIG. 7

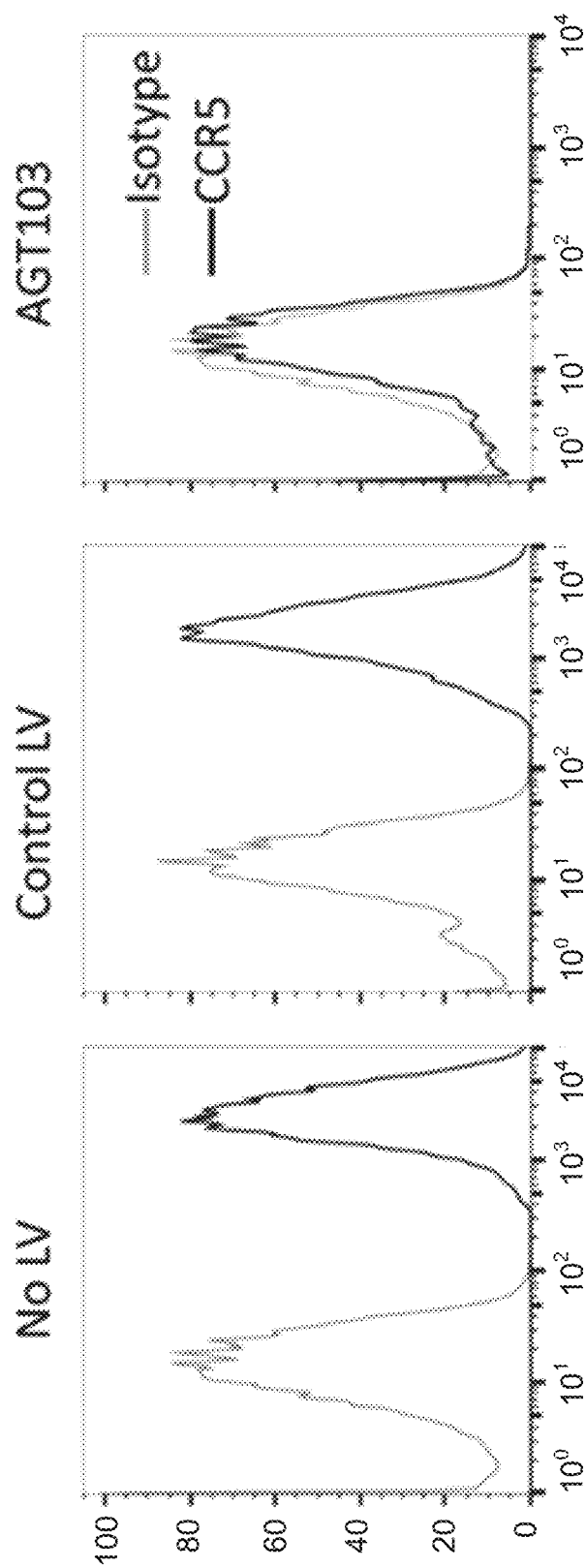


FIG. 8A

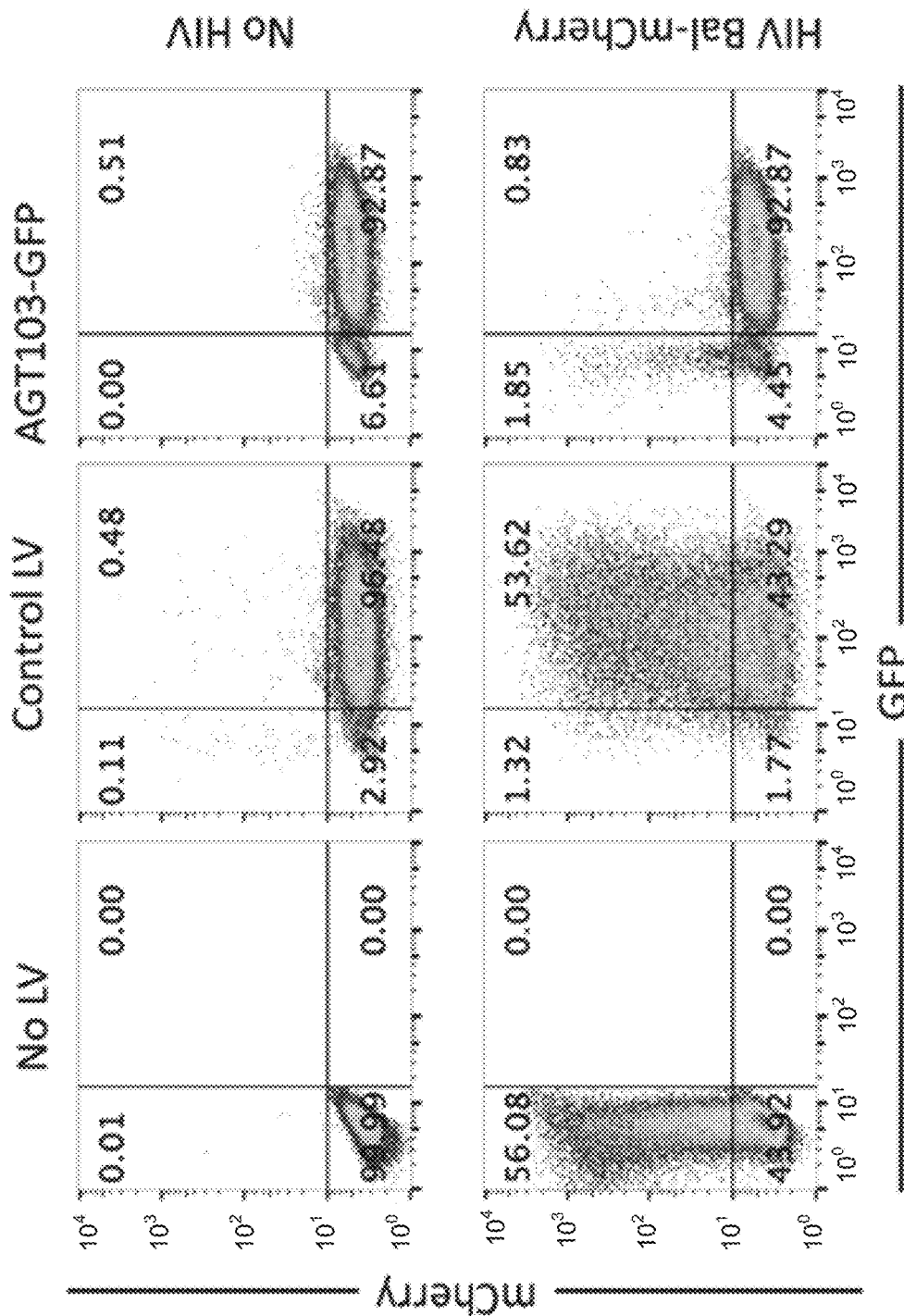


FIG. 8B

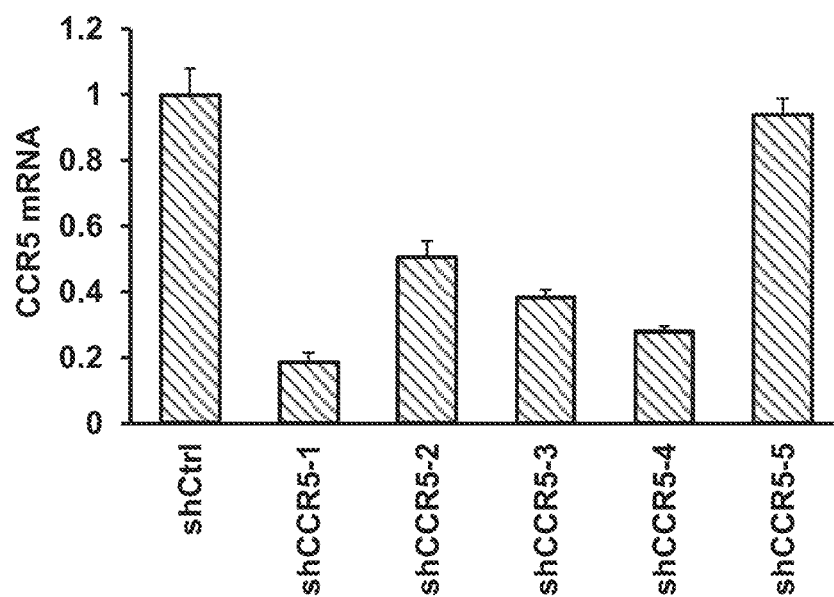


FIG. 9A

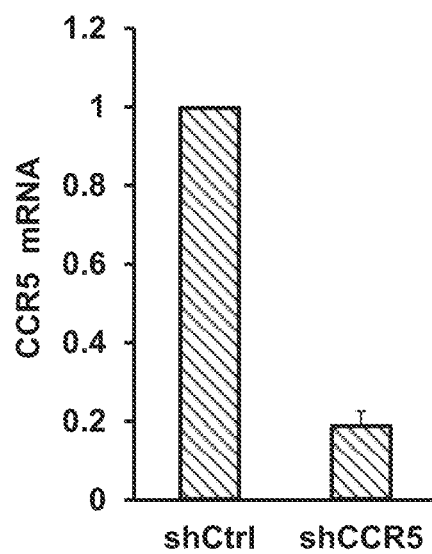


FIG. 9B

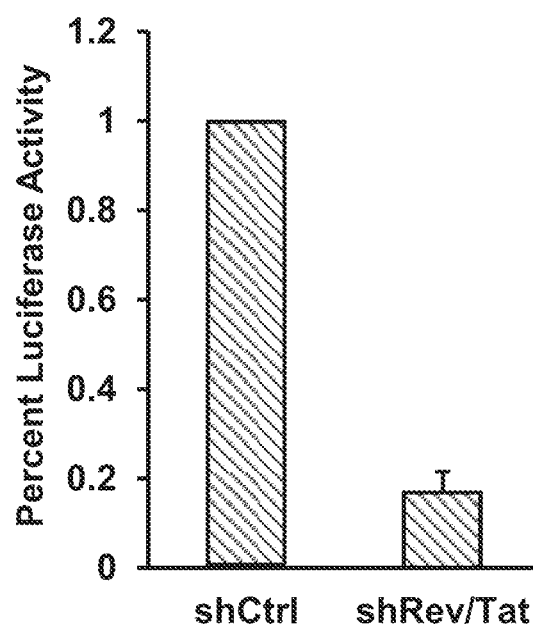


FIG. 10A

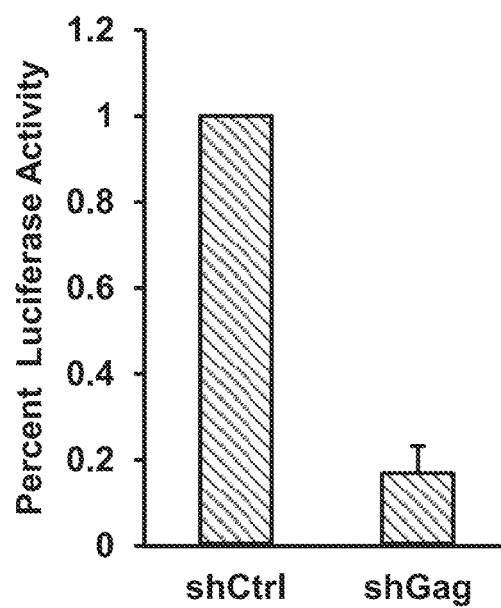


FIG. 10B

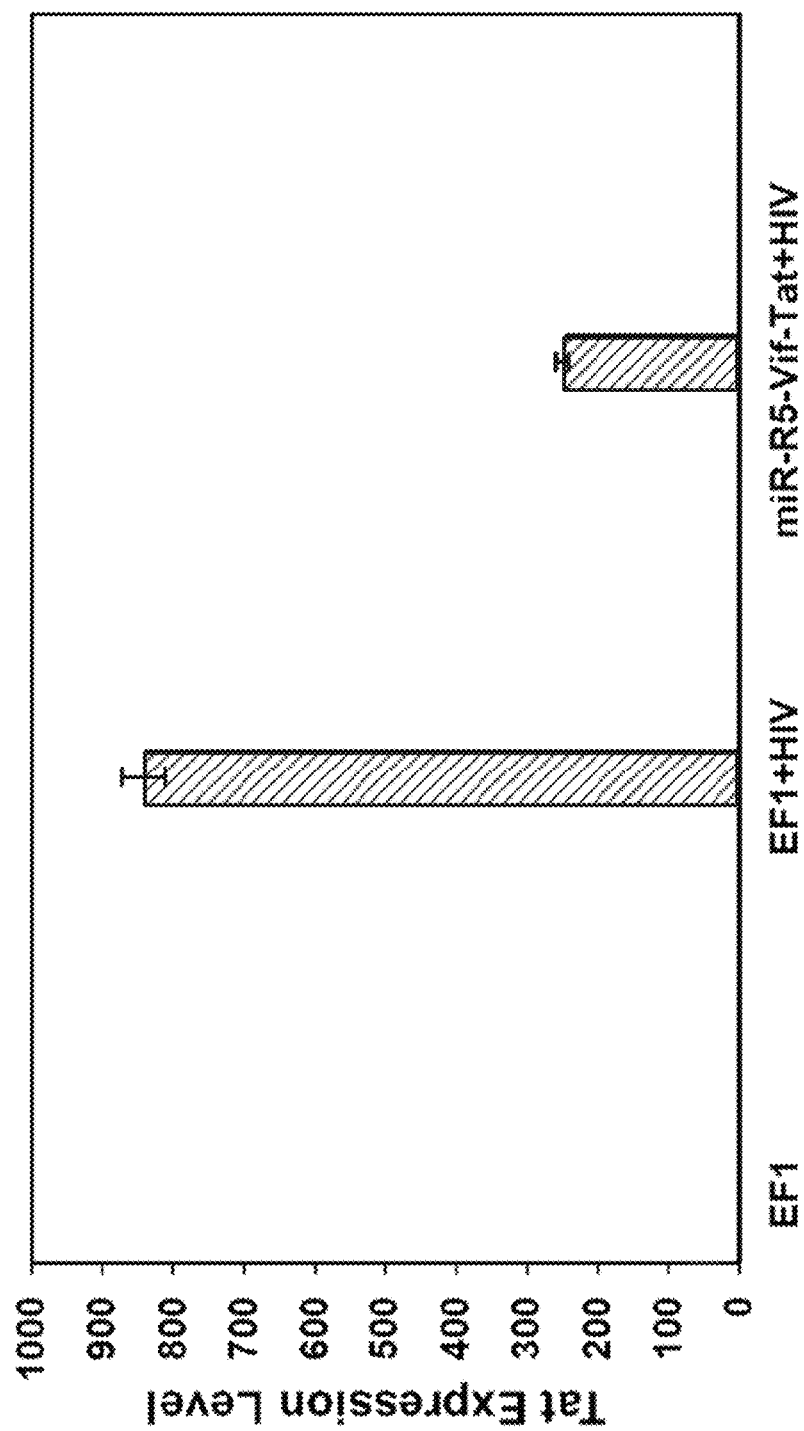


FIG. 11

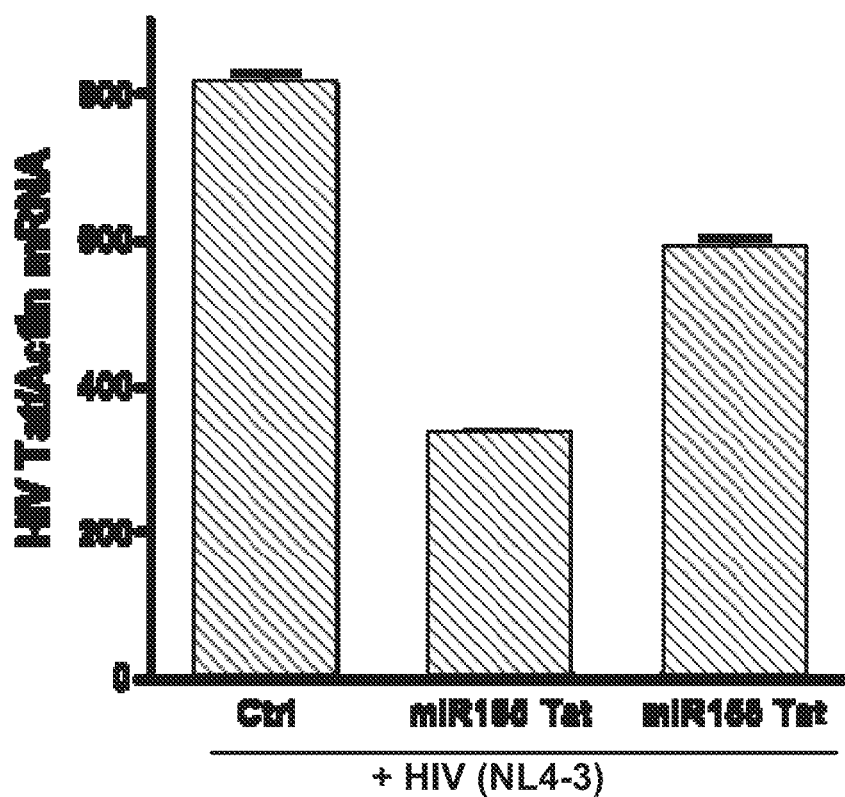


FIG. 12A

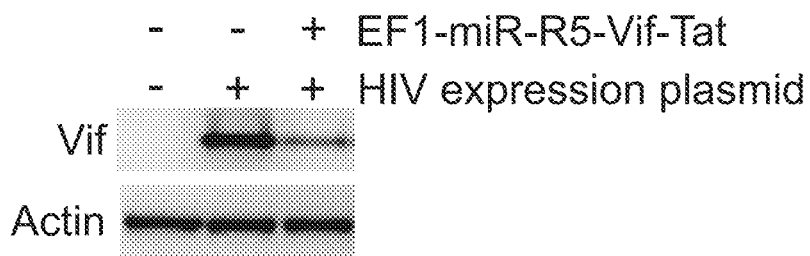


FIG. 12B

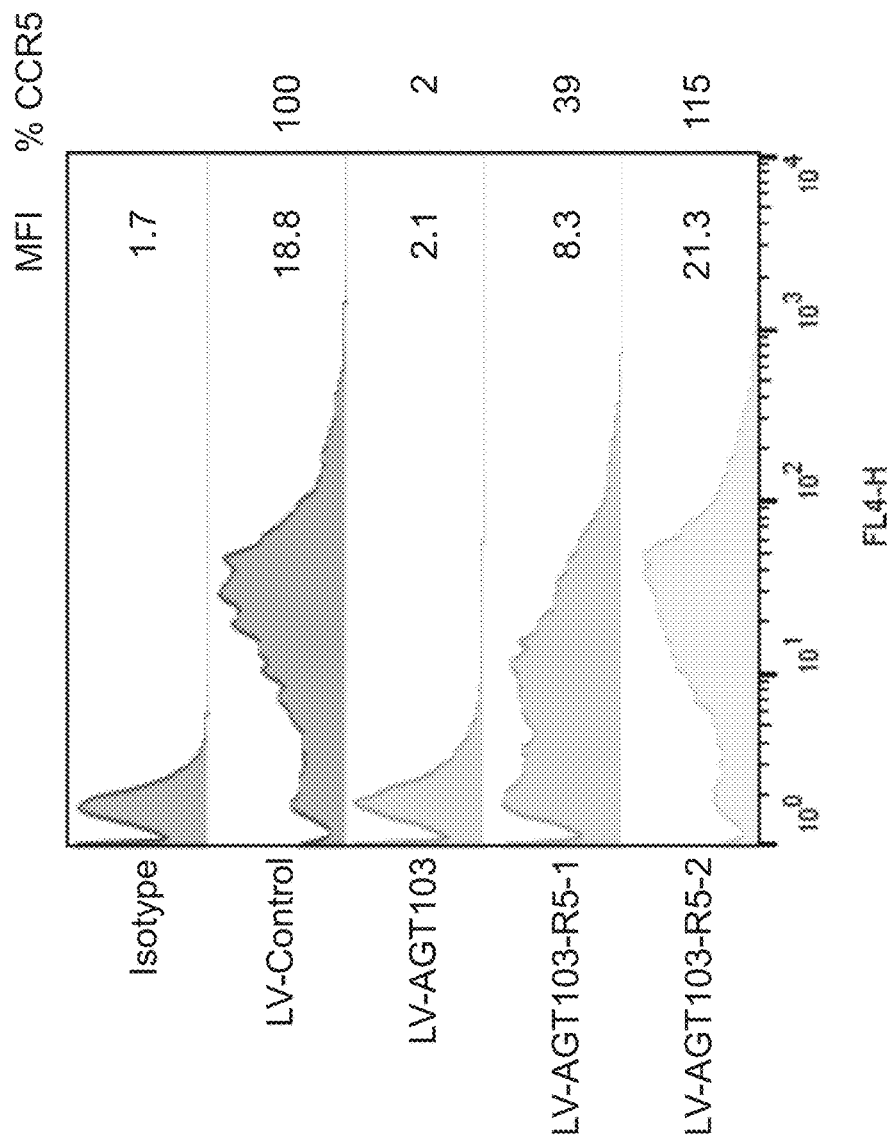


FIG. 13

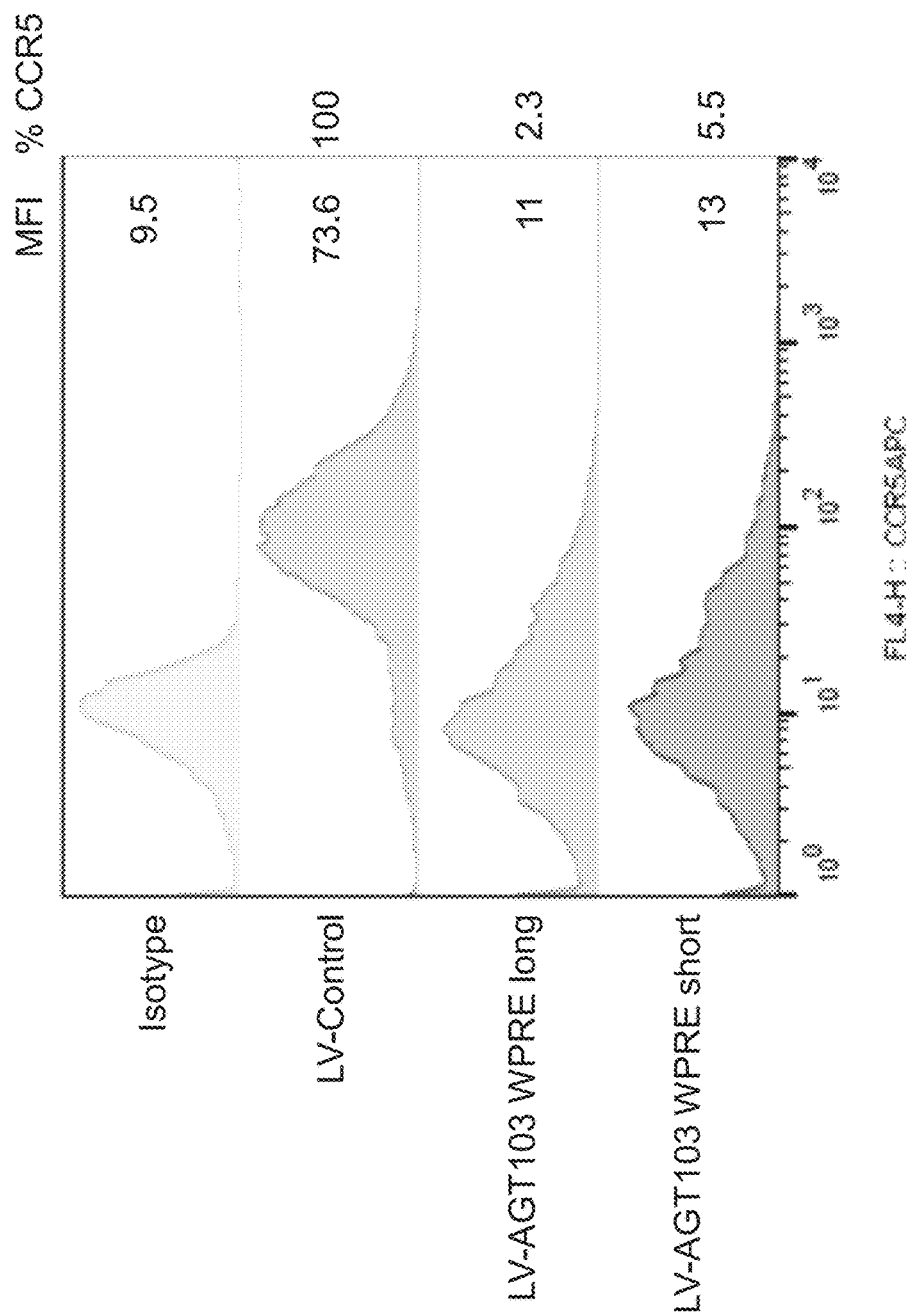
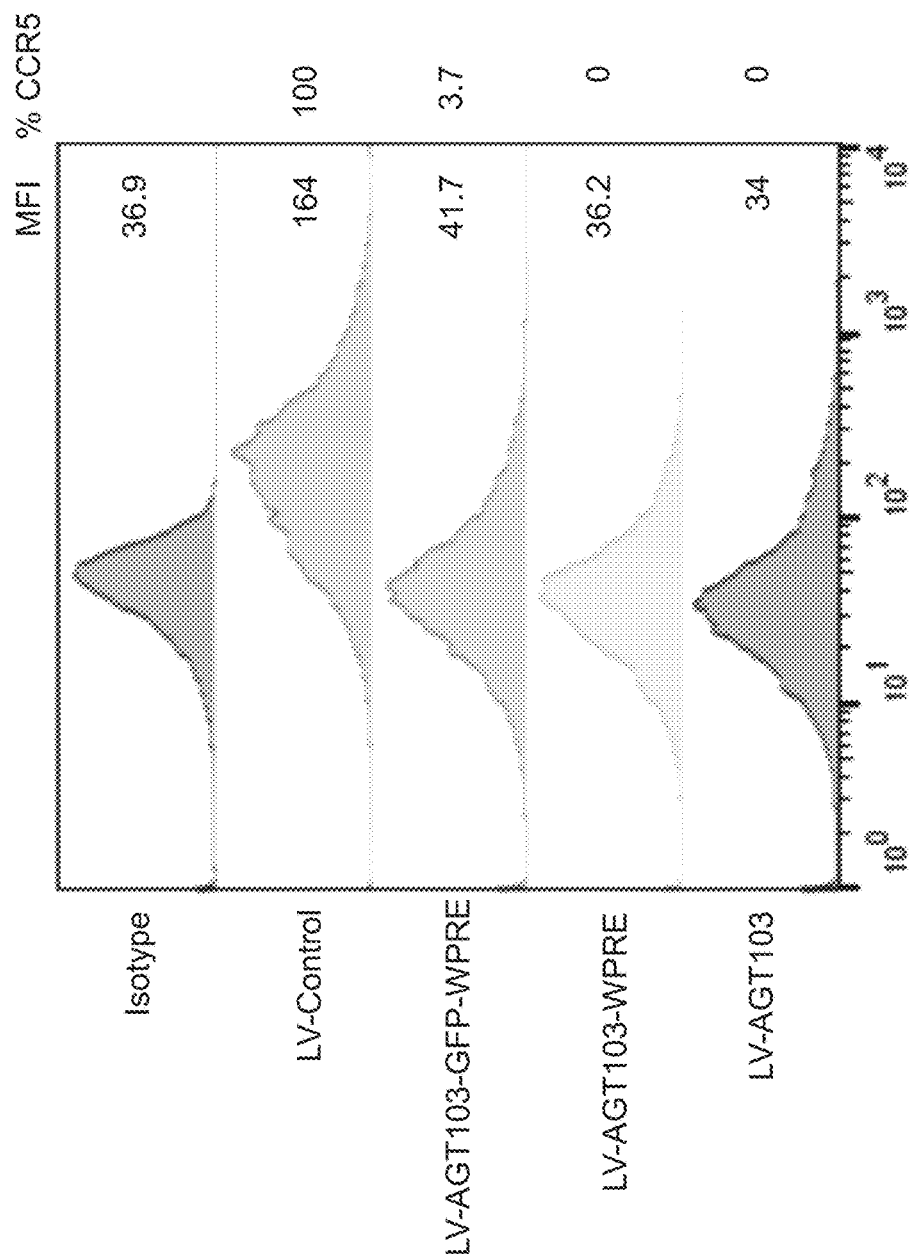


FIG. 14



FL4-H :: CCR5 APC

FIG. 15

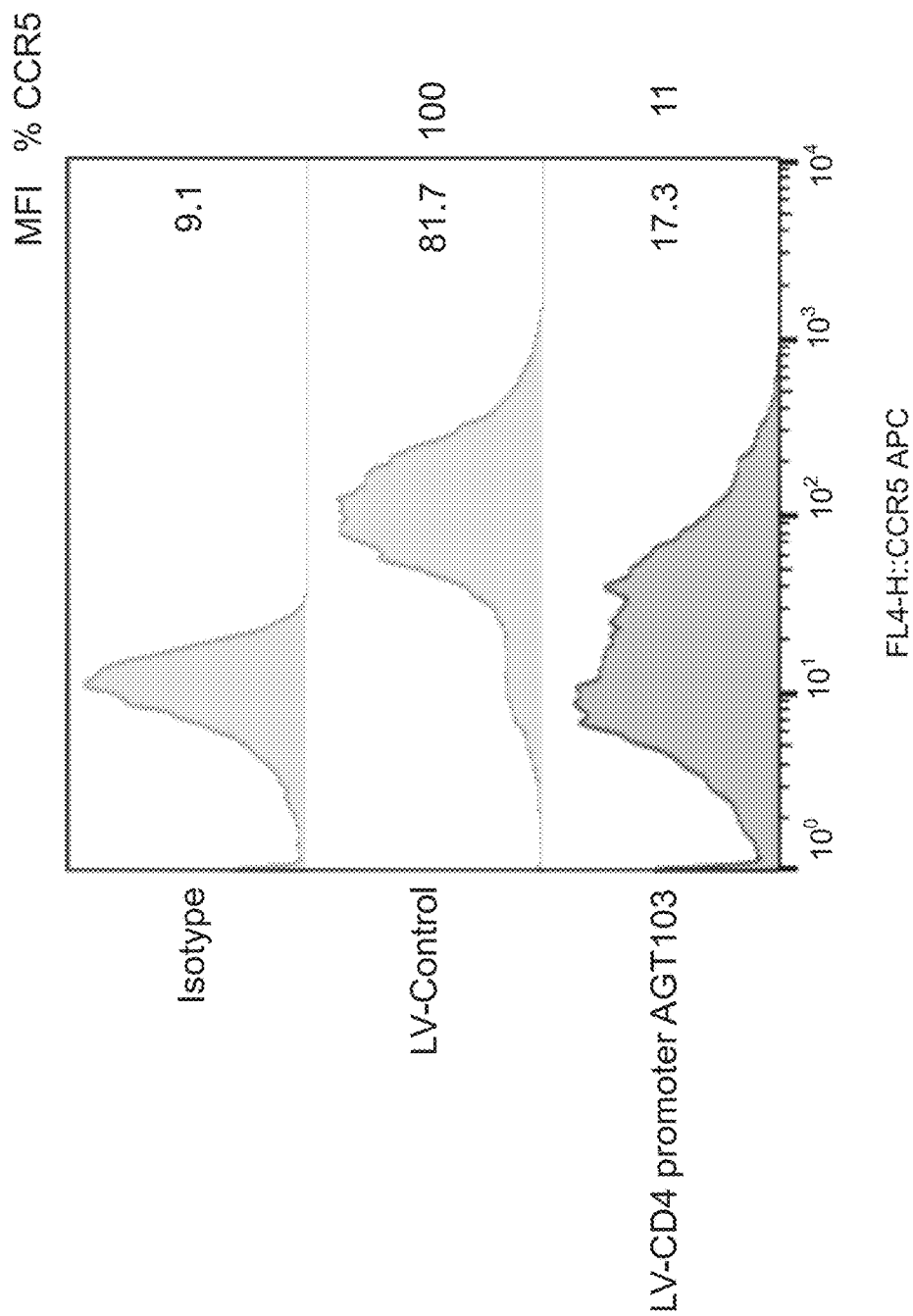


FIG. 16

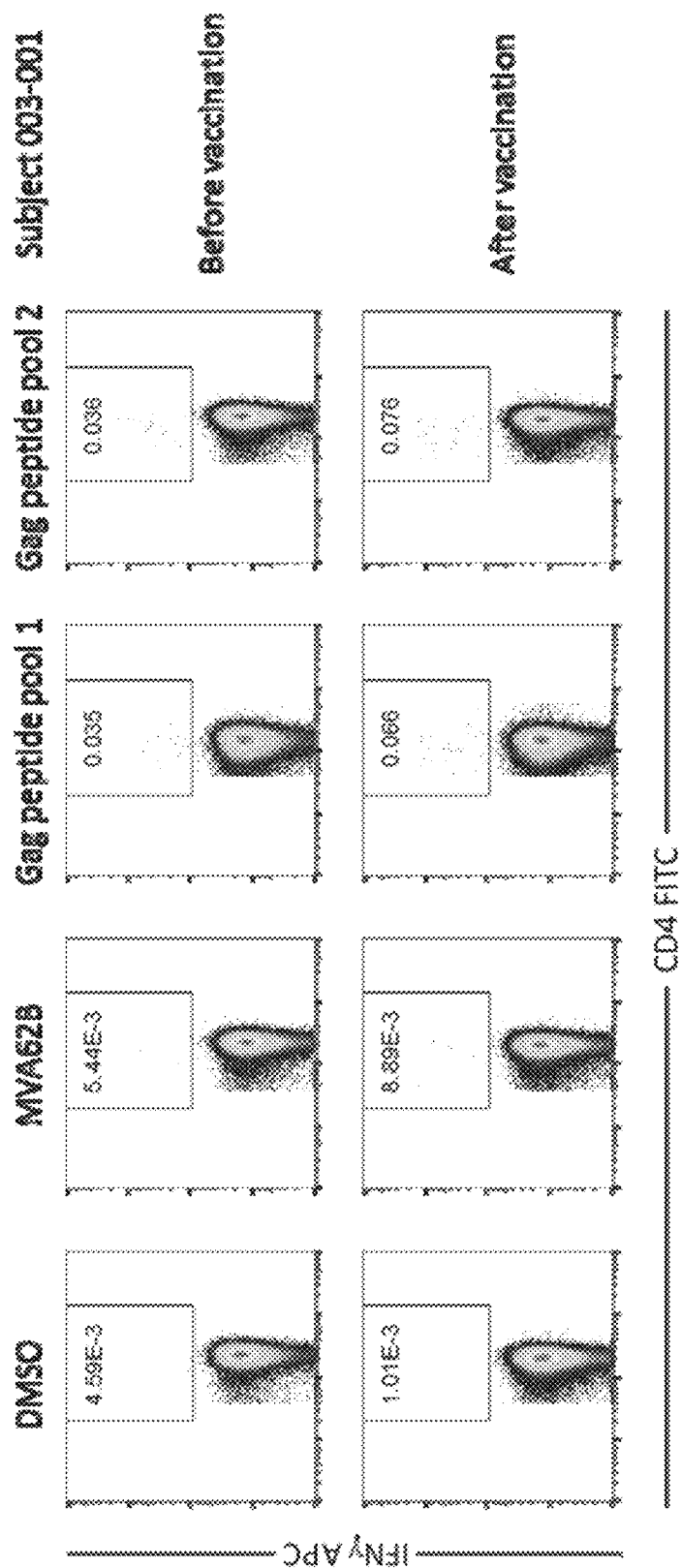
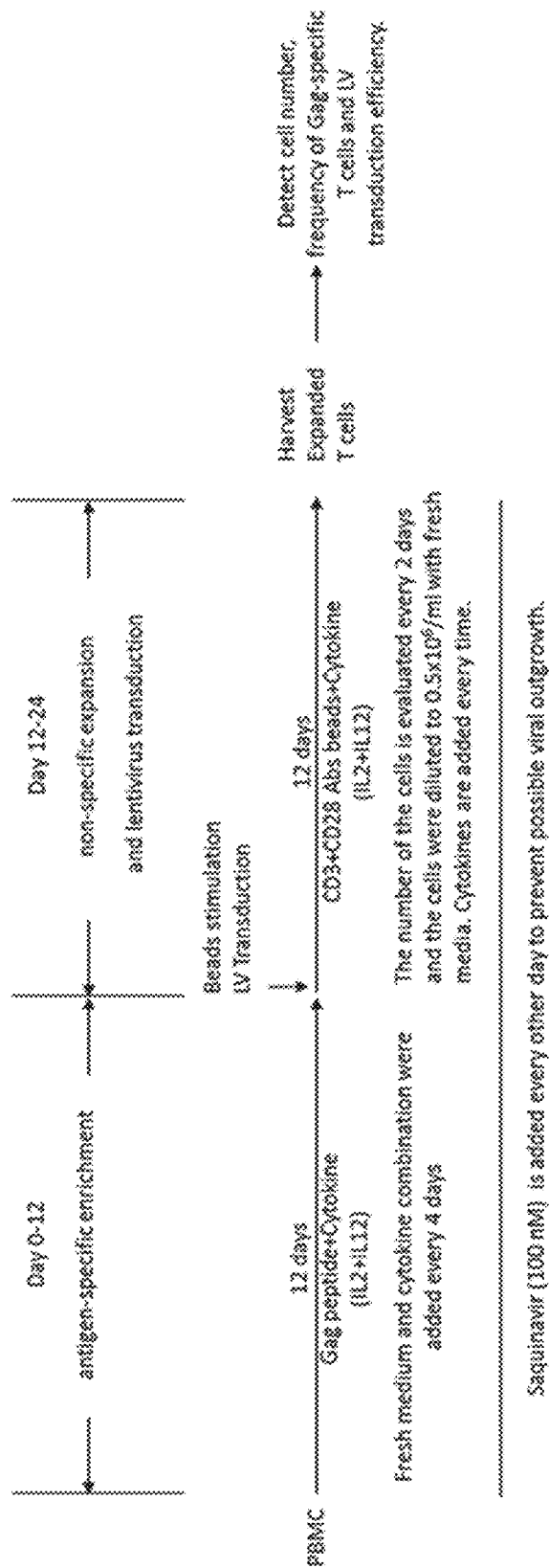


FIG. 17



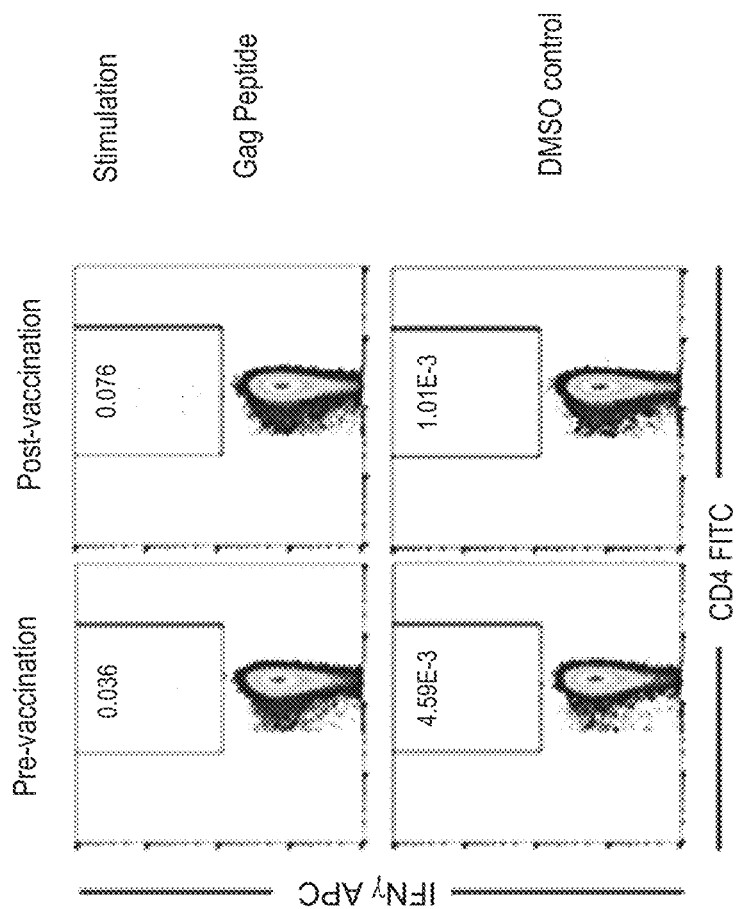


FIG. 18B

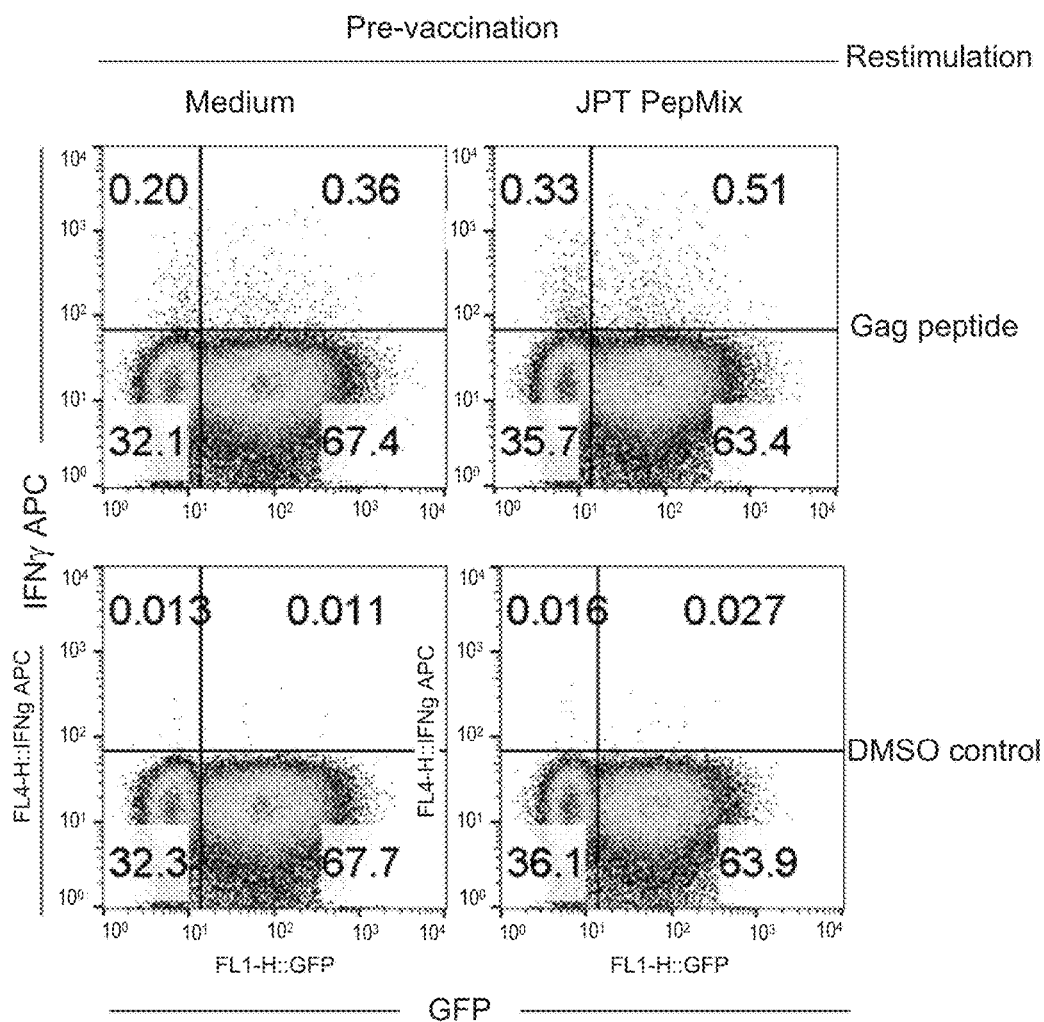


FIG. 18C

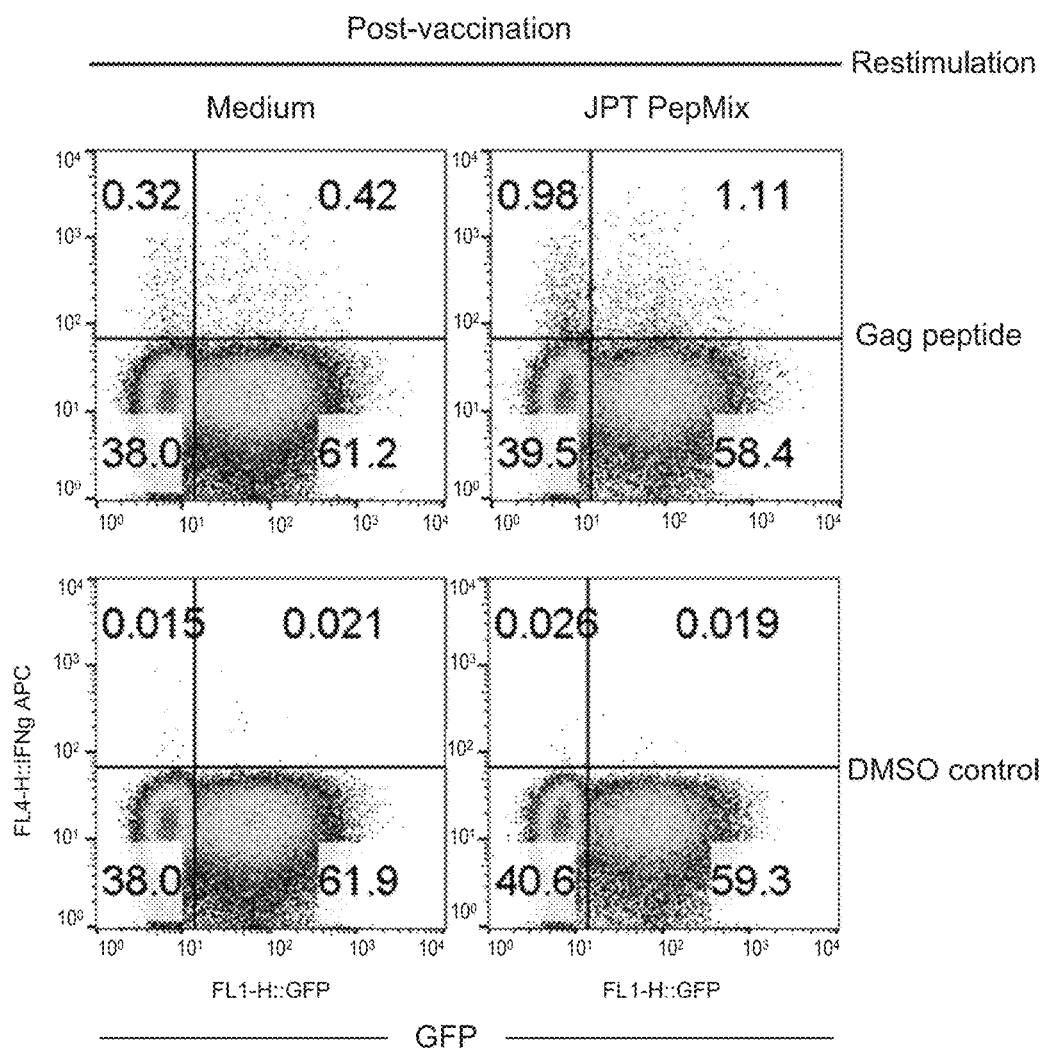


FIG. 18C CONTINUED

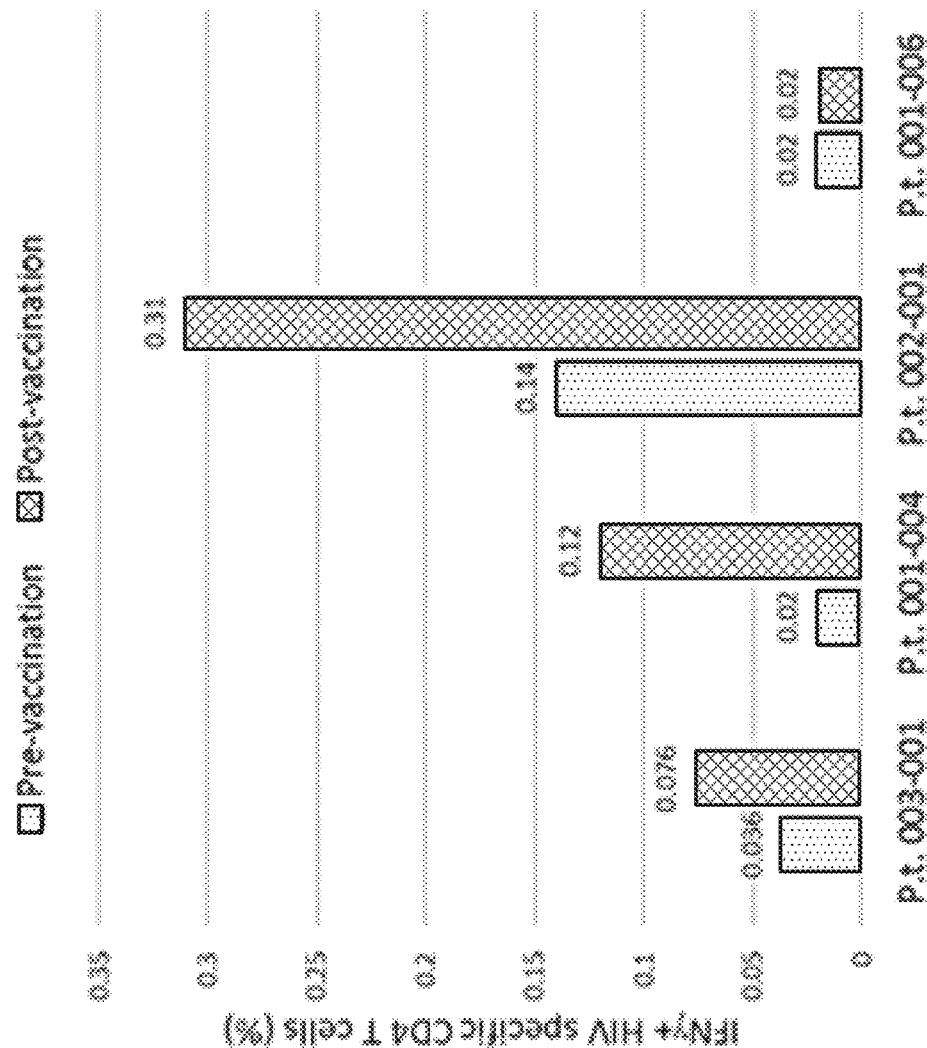


FIG. 18D

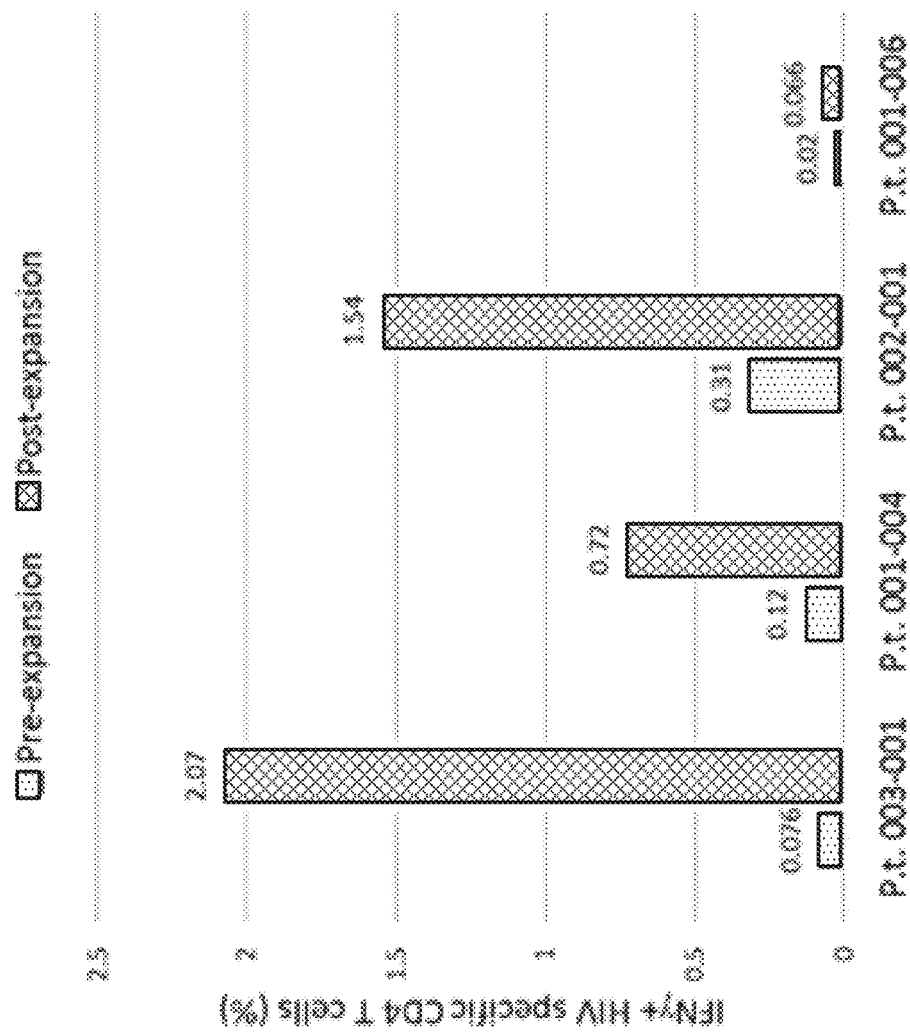


FIG. 18E

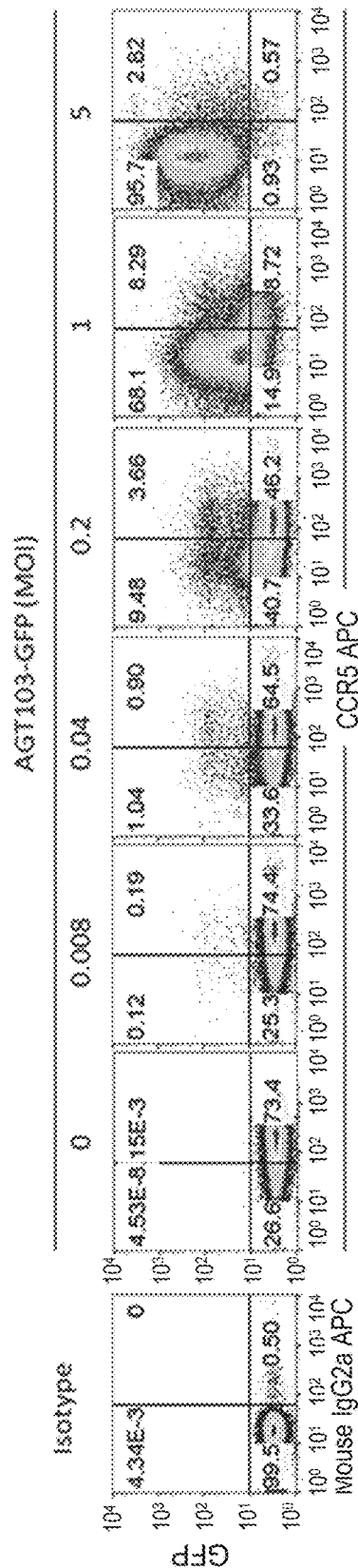
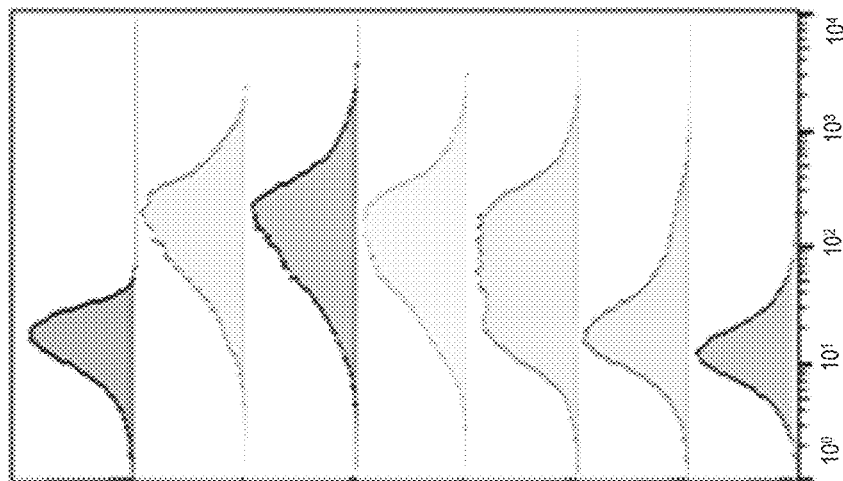


FIG. 19A

Sample Name	Geometric Mean : FL4-H
ISO 001	12.9
LV/MOI=0.002	124
LV/MOI=0.008.003	121
LV/MOI=0.04.004	80.2
LV/MOI=0.2.005	58.4
LV/MOI=1.006	22.5
LV/MOI=5.007	13.0



FL4-H::CCR5 APC

FIG. 19B

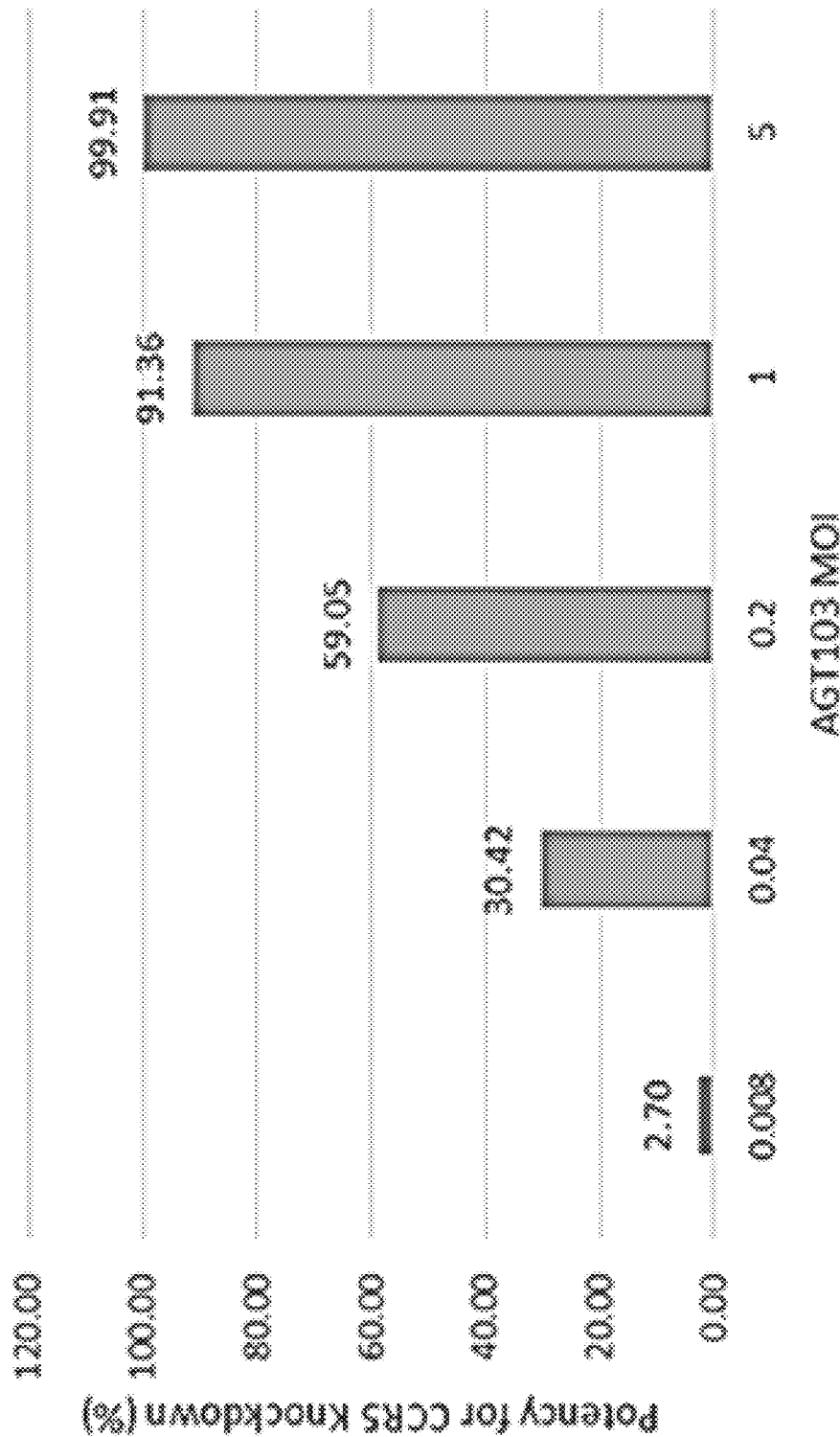


FIG. 19C

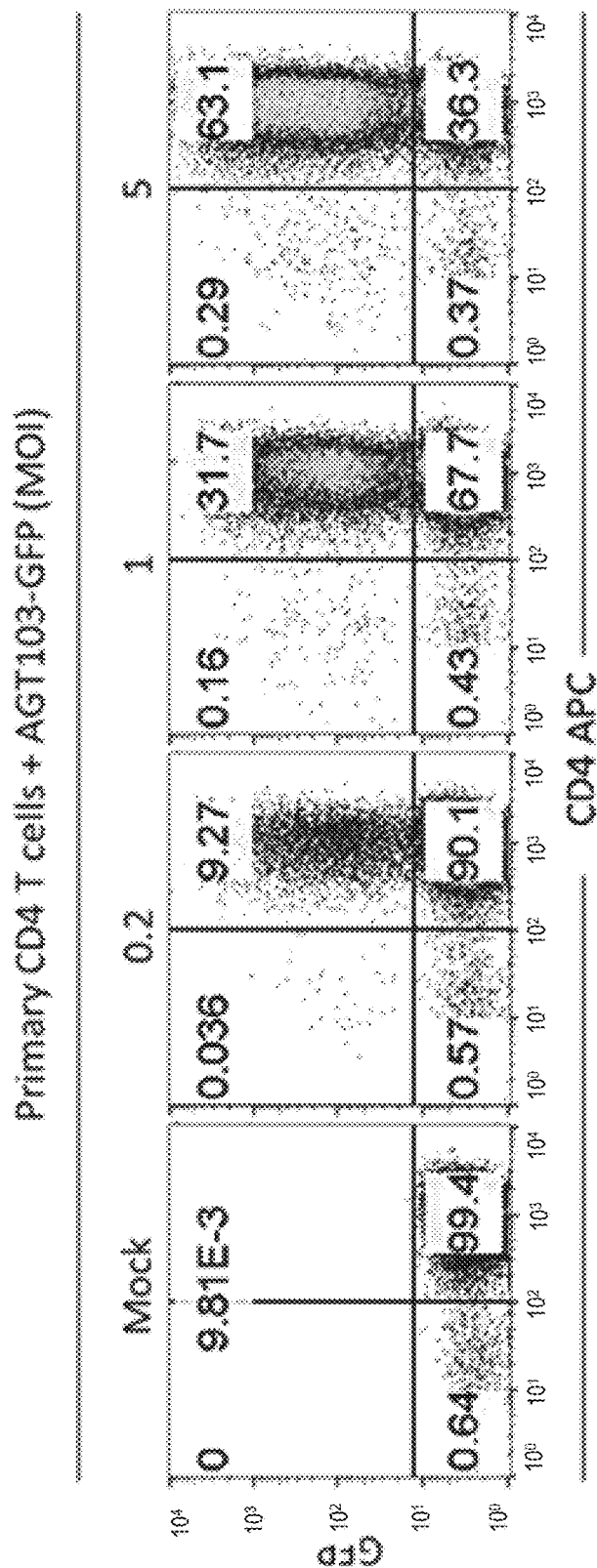


FIG. 20A

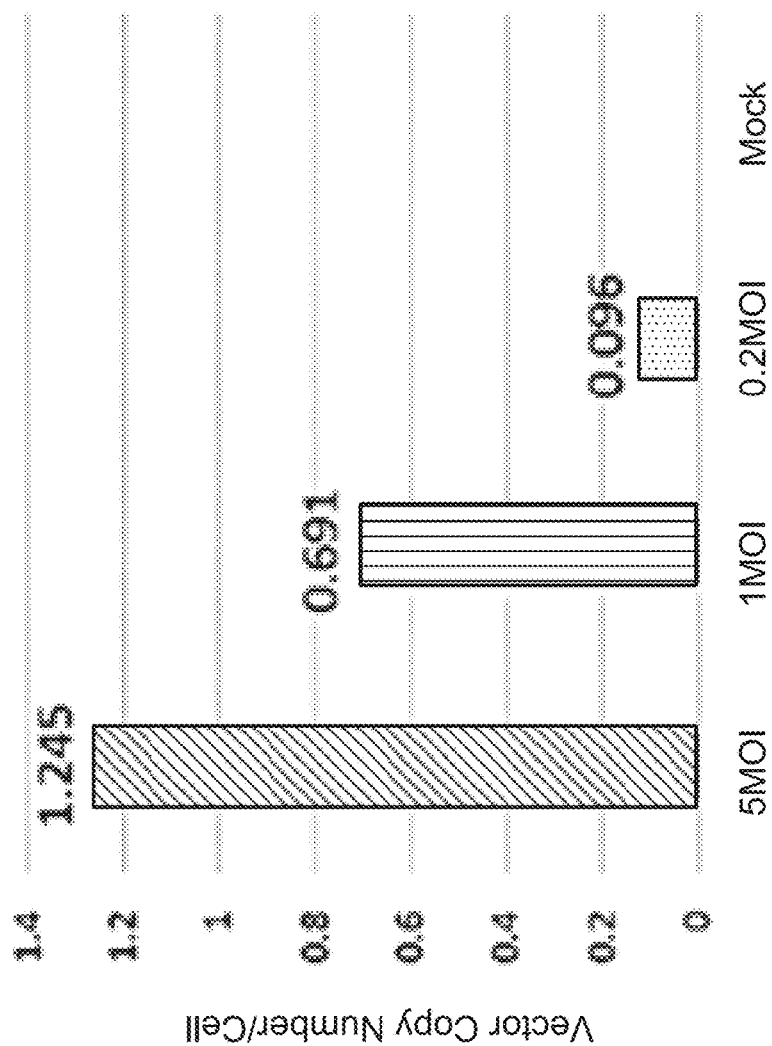


FIG. 20B

C8166 cells infected by supernatant from HIV-infected Primary CD4 T cells

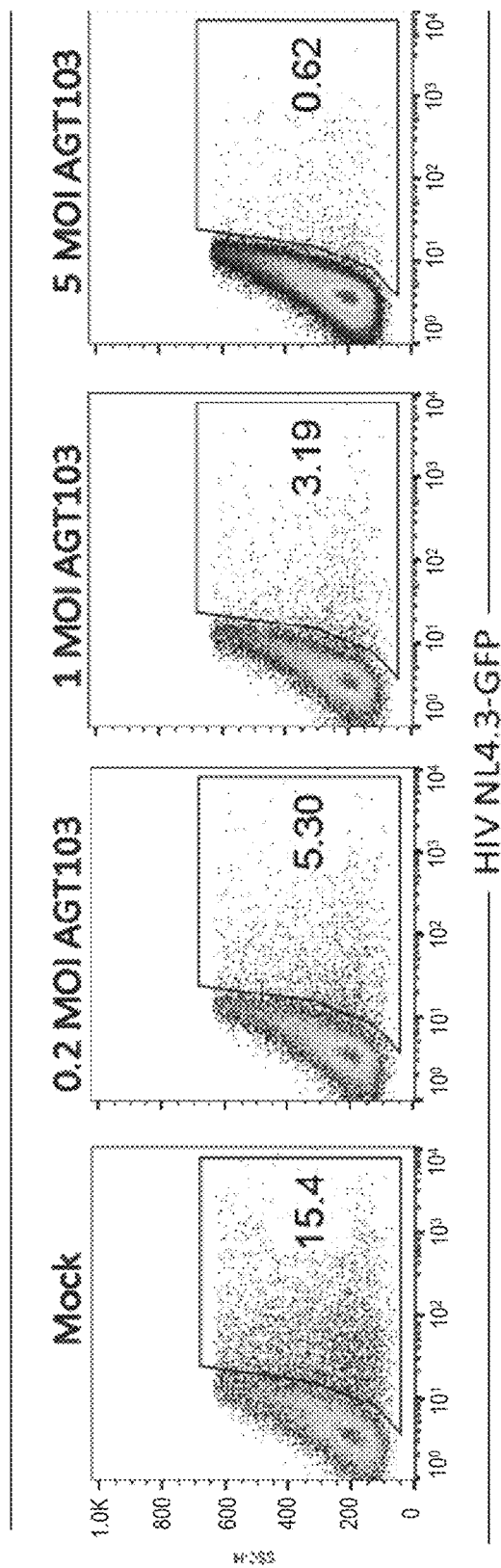


FIG. 21

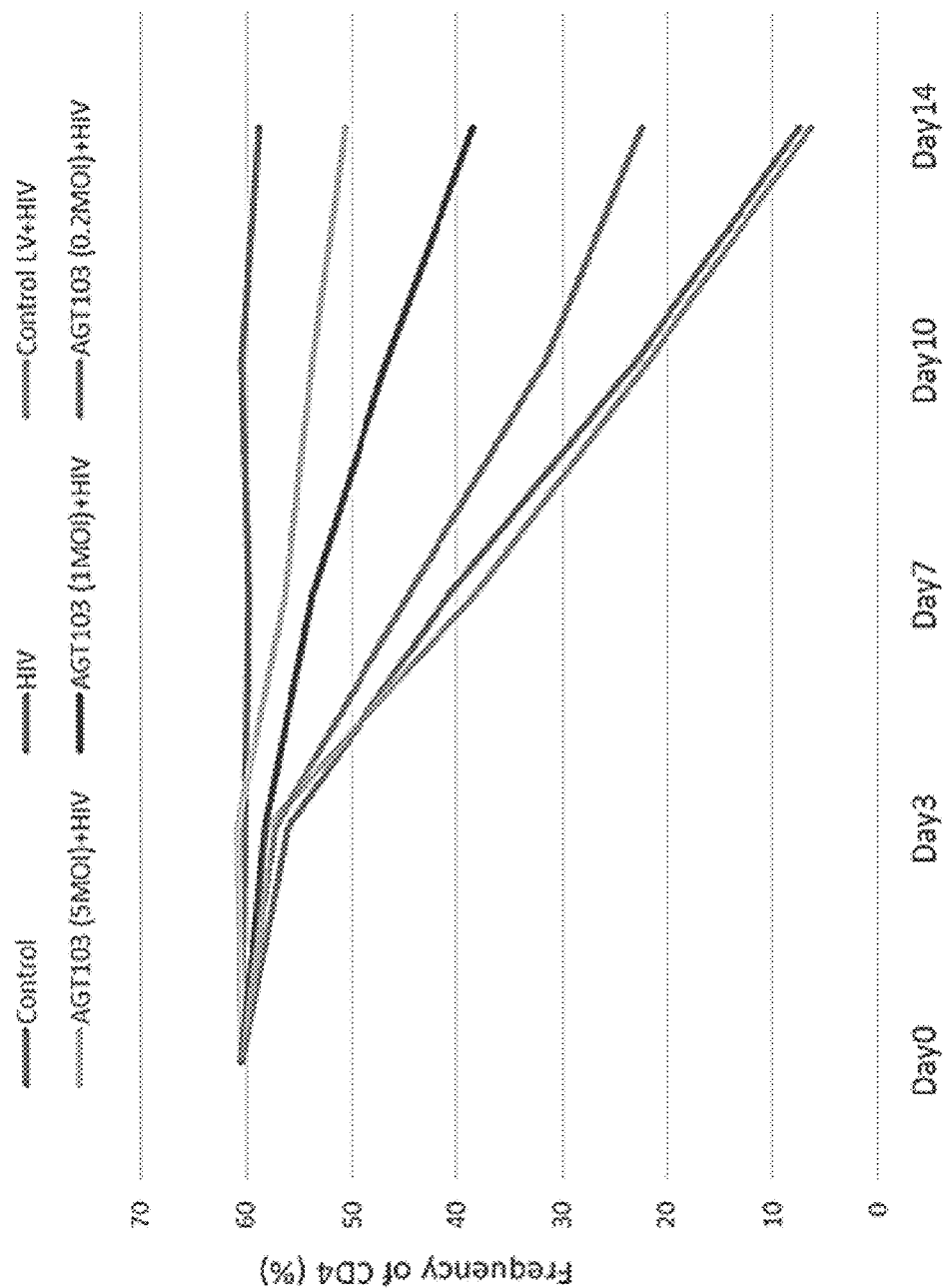


FIG. 22

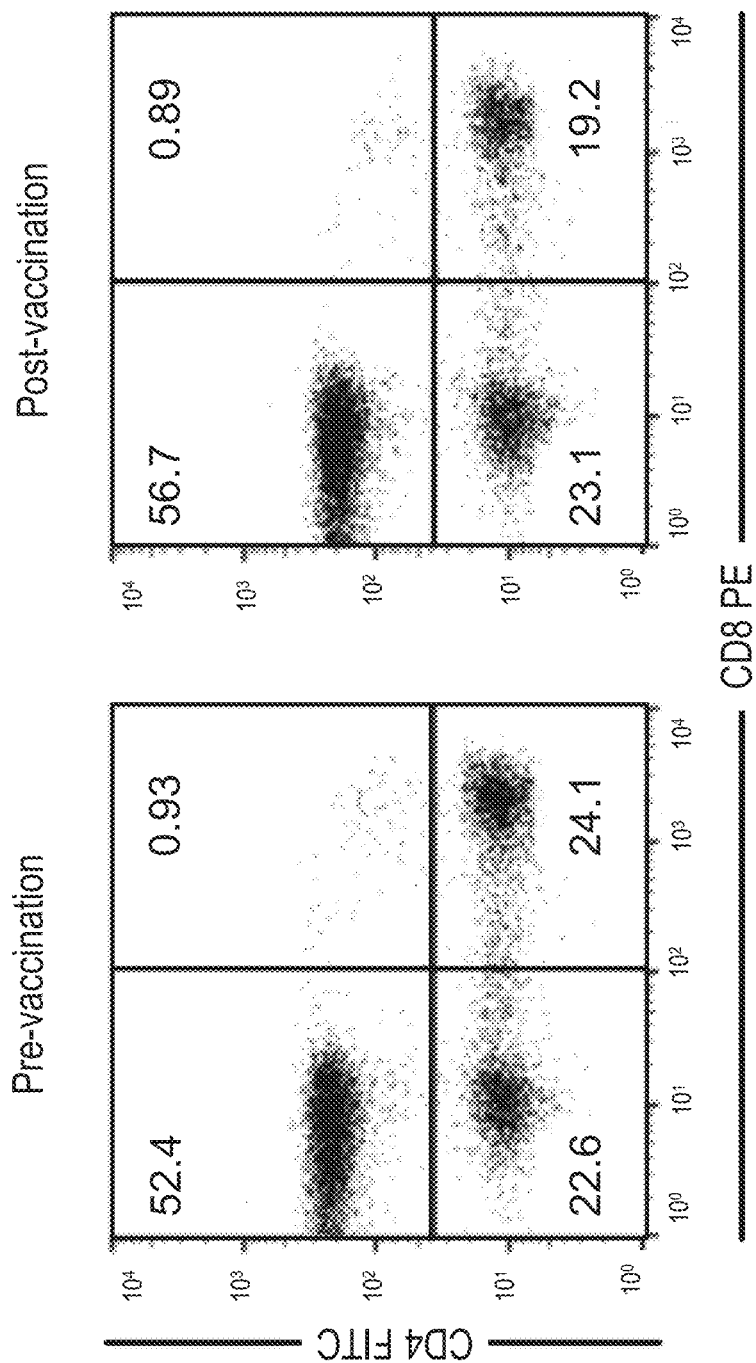


FIG. 23A

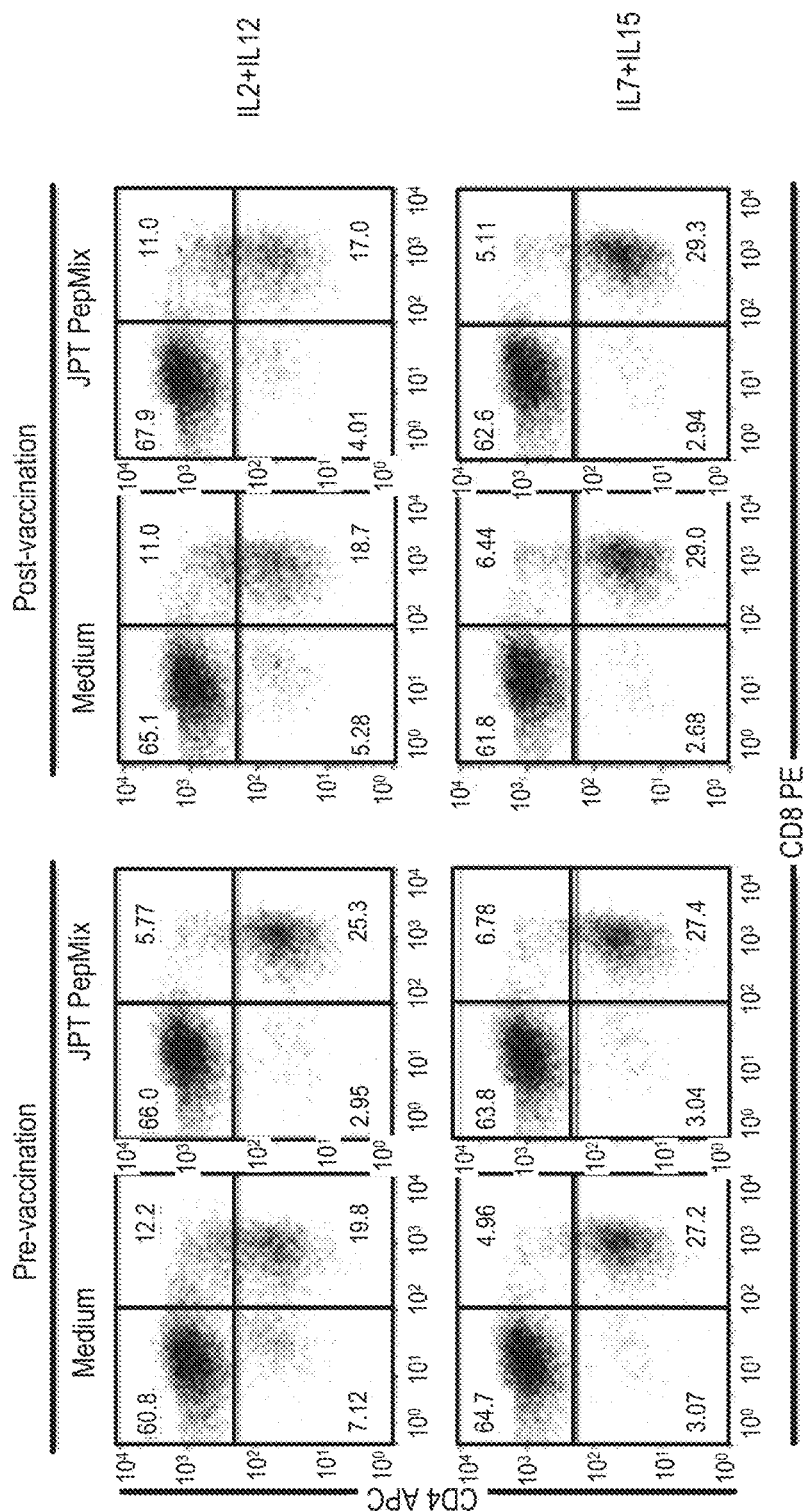


FIG. 23B

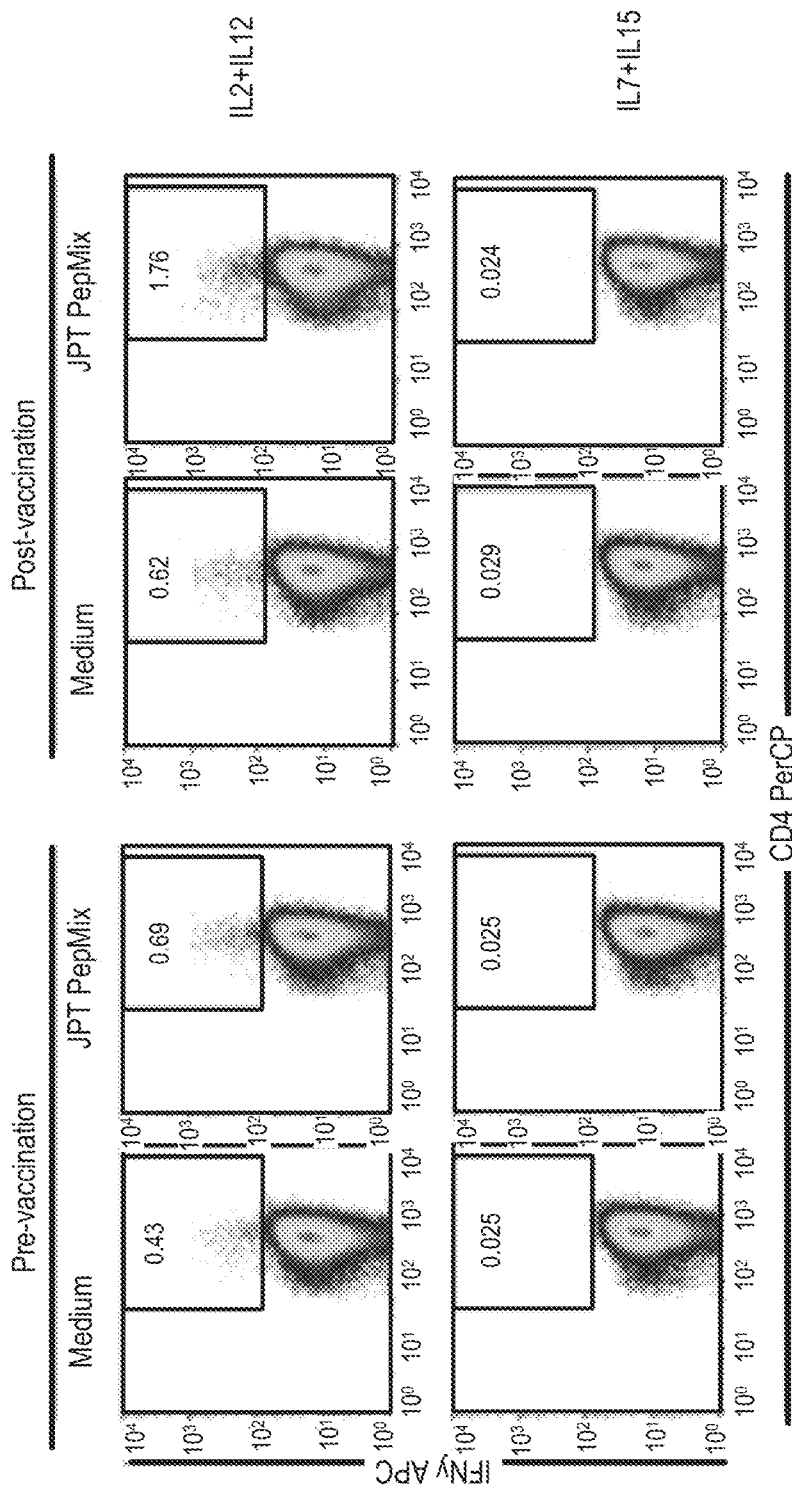


FIG. 23C

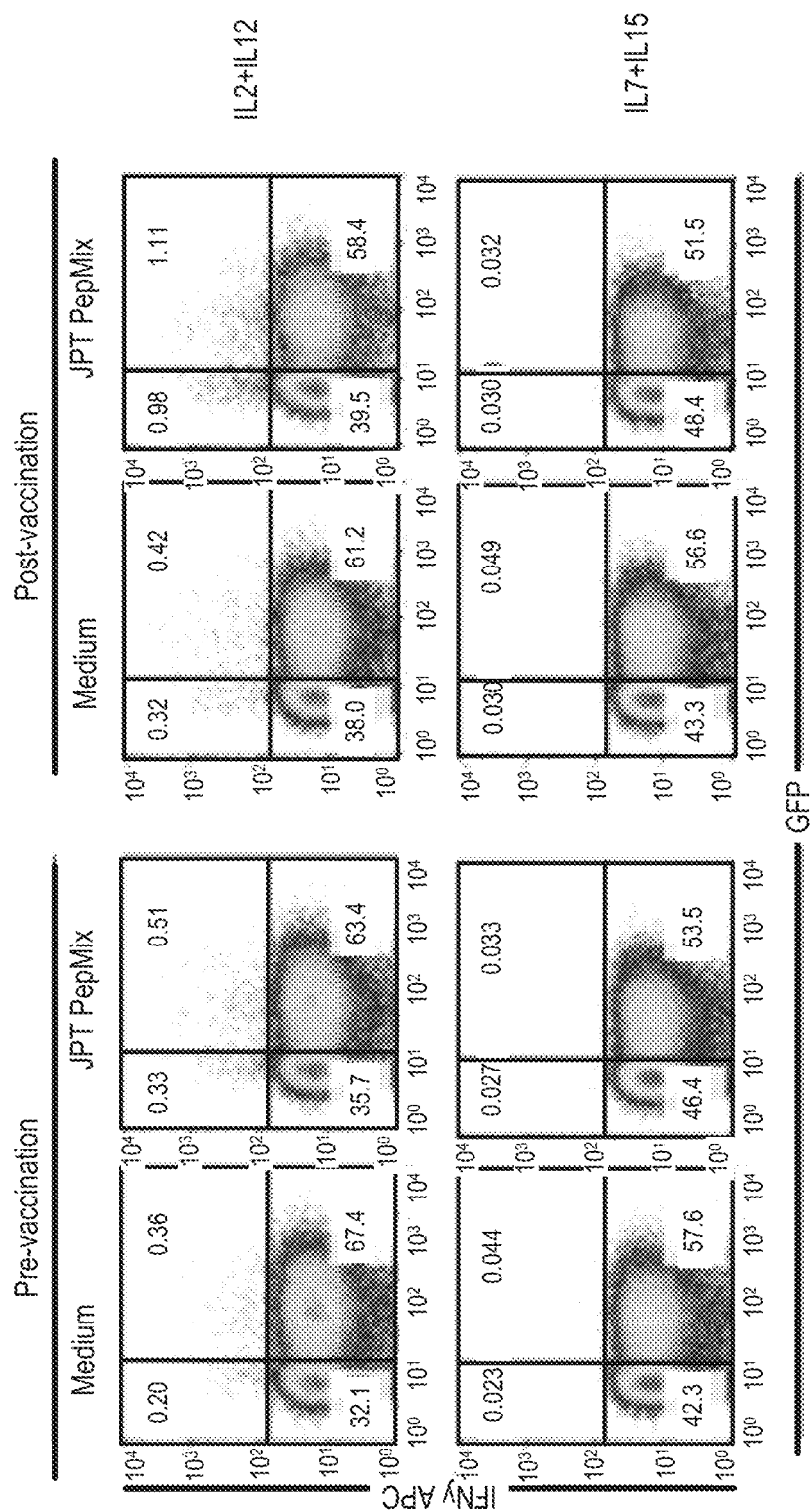


FIG. 23D

HIV PRE-IMMUNIZATION AND IMMUNOTHERAPY

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a continuation of U.S. patent application Ser. No. 16/011,550 filed on Jun. 18, 2018, entitled "HIV PRE-IMMUNIZATION AND IMMUNOTHERAPY" which is a continuation of U.S. patent application Ser. No. 15/668,223 filed on Aug. 3, 2017, entitled "HIV PRE-IMMUNIZATION AND IMMUNOTHERAPY" which is a continuation of International Application No. PCT/US17/13019 filed on Jan. 11, 2017, entitled "HIV PRE-IMMUNIZATION AND IMMUNOTHERAPY" which claims priority to: U.S. Provisional Patent Application No. 62/360,185 filed on Jul. 8, 2016 entitled "HIV PRE-IMMUNIZATION AND IMMUNOTHERAPY", U.S. Provisional Patent Application No. 62/385,864 filed on Sep. 9, 2016 entitled "HIV PRE-IMMUNIZATION AND IMMUNOTHERAPY", and U.S. Provisional Patent Application No. 62/409,270 filed on Oct. 17, 2016 entitled "HIV PRE-IMMUNIZATION AND IMMUNOTHERAPY," the disclosures of which are incorporated herein by reference.

FIELD OF THE INVENTION

The present invention relates generally to the field of immunization and immunotherapy for the treatment and prevention of HIV. In particular, the disclosed methods of treatment and prevention relate to the administration of viral vectors and systems for the delivery of genes and other therapeutic, diagnostic, or research uses.

BACKGROUND OF THE INVENTION

Combination antiretroviral therapy (cART) (also known as Highly Active Antiretroviral Therapy or HAART) limits HIV-1 replication and retards disease progression, but drug toxicities and the emergence of drug-resistant viruses are challenges for long-term control in HIV-infected persons. Additionally, traditional antiretroviral therapy, while successful at delaying the onset of AIDS or death, has yet to provide a functional cure. Alternative treatment strategies are needed.

Intense interest in immunotherapy for HIV infection has been precipitated by emerging data indicating that the immune system has a major, albeit usually insufficient, role in limiting HIV replication. Virus-specific T-helper cells, which are critical to maintenance of cytolytic T cell (CTL) function, likely play a role. Viremia is also influenced by neutralizing antibodies, but they are generally low in magnitude in HIV infection and do not keep up with evolving viral variants in vivo.

Together this data indicates that increasing the strength and breadth of HIV-specific cellular immune responses might have a clinical benefit through so-called HIV immunotherapy. Some studies have tested vaccines against HIV, but success has been limited to date. Additionally, there has been interest in augmenting HIV immunotherapy by utilizing gene therapy techniques, but as with other immunotherapy approaches, success has been limited.

Viral vectors can be used to transduce genes into target cells owing to specific virus envelope-host cell receptor interactions and viral mechanisms for gene expression. As a result, viral vectors have been used as vehicles for the transfer of genes into many different cell types including

whole T cells or other immune cells as well as embryos, fertilized eggs, isolated tissue samples, tissue targets in situ and cultured cells. The ability to introduce and express foreign or altered genes in a cell is useful for therapeutic interventions such as gene therapy, somatic cell reprogramming of induced pluripotent stem cells, and various types of immunotherapy.

Gene therapy is one of the ripest areas of biomedical research with the potential to create new therapeutics that may involve the use of viral vectors. In view of the wide variety of potential genes available for therapy, an efficient means of delivering these genes is needed to fulfill the promise of gene therapy as a means of treating infectious and non-infectious diseases. Several viral systems including murine retrovirus, adenovirus, parvovirus (adeno-associated virus), vaccinia virus, and herpes virus have been proposed as therapeutic gene transfer vectors.

There are many factors that must be considered when developing viral vectors, including tissue tropism, stability of virus preparations, stability and control of expression, genome packaging capacity, and construct-dependent vector stability. In addition, in vivo application of viral vectors is often limited by host immune responses against viral structural proteins and/or transduced gene products.

Thus, toxicity and safety are key hurdles that must be overcome for viral vectors to be used in vivo for the treatment of subjects. There are numerous historical examples of gene therapy applications in humans that have met with problems associated with the host immune responses against the gene delivery vehicles or the therapeutic gene products. Viral vectors (e.g., adenovirus) which co-transduce several viral genes together with one or more therapeutic gene(s) are particularly problematic.

Although lentiviral vectors do not generally induce cytotoxicity and do not elicit strong host immune responses, some lentiviral vectors such as HIV-1, which carry several immunostimulatory gene products, have the potential to cause cytotoxicity and induce strong immune responses in vivo. However, this may not be a concern for lentiviral derived transducing vectors that do not encode multiple viral genes after transduction. Of course, this may not always be the case, as sometimes the purpose of the vector is to encode a protein that will provoke a clinically useful immune response.

Another important issue related to the use of lentiviral vectors is that of possible cytopathogenicity upon exposure to some cytotoxic viral proteins. Exposure to certain HIV-1 proteins may induce cell death or functional unresponsiveness in T cells. Likewise, the possibility of generating replication-competent, virulent virus by recombination is often a concern. Accordingly, there remains a need for improved treatments of HIV.

SUMMARY OF THE INVENTION

In one aspect, a method of treating cells infected with HIV is provided. The method variously includes contacting peripheral blood mononuclear cells (PBMC) isolated from a subject infected with HIV with a therapeutically effective amount of a stimulatory agent, wherein the contacting is carried out ex vivo; transducing the PBMC ex vivo with a viral delivery system encoding at least one genetic element; and culturing the transduced PBMC for a sufficient period of time to ensure adequate transduction. In embodiments, the transduced PBMC may be cultured from about 1 to about 35 days. The method may further include infusing the transduced PBMC into a subject. The subject may be a human.

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The stimulatory agent may include any agent suitable for stimulating a T cell response in a subject. In embodiments, the stimulatory agent is a peptide or mixture of peptides, and in embodiments includes a gag peptide. The stimulatory agent may also include a vaccine. The vaccine may be a HIV vaccine, and in embodiments, the HIV vaccine is a MVA/HIV62B vaccine or a variant thereof. In embodiments, the viral delivery system includes a lentiviral particle. In embodiments, the at least one genetic element includes a small RNA capable of inhibiting production of chemokine receptor CCR5. In further embodiments, the at least one genetic element includes at least one small RNA capable of targeting an HIV RNA sequence. In further embodiments, the at least one genetic element may include a small RNA capable of inhibiting production of chemokine receptor CCR5 and at least one small RNA capable of targeting an HIV RNA sequence. The HIV RNA sequence includes any HIV sequence suitable for targeting by a viral delivery system. In embodiments, the HIV RNA sequence includes one or more of a HIV Vif sequence, a HIV Tat sequence, or a variant thereof. The at least one genetic element includes any genetic element capable of being expressed by a viral delivery system. In embodiments, the at least one genetic element includes a microRNA or a shRNA. In further embodiments, the at least one genetic element comprises a microRNA cluster.

In another aspect, the at least one genetic element includes a microRNA having at least 80%, or at least 85%, or at least 90%, or at least 95% percent identity with AGGTATATTGCTGTTGACAGTGAGCGACTGTAACTGAGCTT-GCTCTACTGTGAAGCC ACAGATGGGTAGAG-CAAGCACAGTTTACCGCTGCCTACTGCCTCGGACTTCAAGGGG CTT (SEQ ID NO: 1). In a preferred embodiment, the at least one genetic element comprises: AGGTATATTGCTGTTGACAGTGAGCGACTGTAACTGAGCT-TGCTCTACTGTGAAGCC ACAGATGGGTAGAGCAAGCACAGTTTACCGCTGCCTACTGCCTCGGACTTCAAGGGG CTT (SEQ ID NO: 1).

In another aspect, the at least one genetic element includes a microRNA having at least 80%, or at least 85%, or at least 90%, or at least 95% percent identity with CATCTCCATG-GCTGTACCACCTTGTGCGGGGATGTGTACTTCT-GAAGTTGTGTTGAAT CTCATGGAGTTCA-GAAGAACACATCCGCACTGACATTTGGTATCTTT CATCTGACCA (SEQ ID NO: 2); or at least 80%, or at least 85%, or at least 90%, or at least 95% percent identity with GGGCCTGGCTCGAGCAGGGGCGAGGGATTCCGCTTCTTCTGCCATAGCGTGG TCCCTC-CCCTATGGCAGGCAGAGCGGCACCTTCCCTC-CCAATGACCGCGTCTTCGT CG (SEQ ID NO: 3). In a preferred embodiment, the at least one genetic element includes CATCTCCATGGCTGTACCACCTTGTGCGGGGATGTGTACTTCTGAACTTGTGTTGAATCTCATGGAGTTTCAAGAACACATCCGCACTGACATTTGGTATCTTTTATCTGACCA (SEQ ID NO: 2); or GGGCCTGGCTCGAGCAGGGGCGAGGGATTCCGCTTCTTCTGCCATAGCGTGGTCCCTC-CCCTATGGCAGGCAGAGCGGCACCTTCCCTC-CCAAT GACCGCGTCTTCGTG (SEQ ID NO: 3).

In another aspect, the microRNA cluster includes a sequence having at least 80%, or at least 85%, or at least 90%, or at least 95% percent identity with AGGTATATTGCTGTTGACAGTGAGCGACTGTAACTGAGCTT-GCTCTACTGTGAAGCC ACAGATGGGTAGAG-CAAGCACAGTTTACCGCTGCCTACTGCCTCGGACTTCAAGGGG CTTCCTGGGCATCTCCATGGCTGTAC-

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CACCTTGTCGGGGGATGTGTACTTCTGAACTTGTGTTGAATCTCATGGAGTTTCAAGAACACATCCGCACTGACATTTTGGTATCTTTC ATCTGACCA-GCTAGCGGGCCTGGCTCGAGCAGGGGGC-
5 GAGGGATTCCGCTTCTTCTCCTGCCATAGCGTGGTCCCCCTCCCCTATGGCAGGCA-GAAGCGGCACCTTCCCTCCCAATGA CCGCGTCTTCGTC (SEQ ID NO: 31). In a preferred embodiment, the microRNA cluster includes: AGGTATATTGCTGTTGACAGTGAGCGACTGTAACTGAGCTTGTCTCT ACTGTGAAGCCACAGATGGGTAGAGCAAGCACAGTTTACCGCTGCCTACTGCCTCGG ACTTCAAGGGGCTTCCCGGGCATCTCCATGGCTGTACCACTTGTGCGGGGATGTGTA CTCTGAACTTGTGTTGAATCTCATGGAGTTTCAAGAACACATCCGCACTGACATTT
10 TGGTATCTTTCATCTGACCAGCTAGCGGGCCTG-GCTCGAGCAGGGGCGAGGGATTG CGCTTCTTCTGCCATAGCGTGGTCCCCCTCCCCTATGGCAGGCA-GAAGCGGCACCTTC
20 CTTCCCAATGACCGCGTCTTCGTC (SEQ ID NO: 31).

In another aspect, a method of treating HIV infection in a subject is disclosed. The method variously includes immunizing the subject with an effective amount of a first stimulatory agent; removing leukocytes from the subject and obtaining peripheral blood mononuclear cells (PBMC). The method further includes contacting the PBMC ex vivo with a therapeutically effective amount of a second stimulatory agent; transducing the PBMC ex vivo with a viral delivery system encoding at least one genetic element; and culturing the transduced PBMC for a sufficient period of time to ensure adequate transduction. In embodiments, the transduced PBMC may be cultured from about 1 to about 35 days. In embodiments, the method further involves infusing the transduced PBMC into a subject. The subject may be a human. The first and second stimulatory agents may be the same or different. The first and second stimulatory agents may include one or more of a peptide or mixture of peptides. In embodiments, at least one of the first and second stimulatory agents includes a gag peptide. The at least one of the first and second stimulatory agents may include a vaccine. The vaccine may be a HIV vaccine, and in a preferred embodiment, the HIV vaccine is a MVA/HIV62B vaccine or a variant thereof. In a preferred embodiment, the viral delivery system includes a lentiviral particle. In embodiments, the at least one genetic element includes a small RNA capable of inhibiting production of chemokine receptor CCR5. In embodiments, the at least one genetic element includes at least one small RNA capable of targeting an HIV RNA sequence. In embodiments, the at least one genetic element includes a small RNA capable of inhibiting production of chemokine receptor CCR5 and at least one small RNA capable of targeting an HIV RNA sequence. The HIV RNA sequence may include a HIV Vif sequence, a HIV Tat sequence, or a variant thereof. The at least one genetic element may include a microRNA or a shRNA. In a preferred embodiment, the at least one genetic element comprises a microRNA cluster.

In another aspect, the at least one genetic element includes a microRNA having at least 80%, or at least 85%, or at least 90%, or at least 95% percent identity with AGGTATATTGCTGTTGACAGTGAGCGACTGTAACTGAGCTT-GCTCTACTGTGAAGCC ACAGATGGGTAGAG-CAAGCACAGTTTACCGCTGCCTACTGCCTCGGACTTCAAGGGG CTT (SEQ ID NO: 1). In a preferred embodiment, the at least one genetic element comprises: AGGTATATTGCTGTTGACAGTGAGCGACTGTAACTGAGCT-

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TGCTCTACTGTGAAGCC
ACAGATGGGTAGAGCAAGCACAGTTTACCGCTGC-
CTACTGCCTCGGACTTCAAGGGG CTT (SEQ ID NO:
1).

In another aspect, the at least one genetic element includes a microRNA having at least 80%, or at least 85%, or at least 90%, or at least 95% percent identity with CATCTCCATG-GCTGTACCACCTTGTTCGGGGGATGTGTACTTCT-
GAACTTGTGTTGAAT CTCATGGAGTTCA-
GAAGAACACATCCGCACTGACATTTTGGTATCTTT
CATCTGACCA (SEQ ID NO: 2); or at least 80%, or at least 85%, or at least 90%, or at least 95% percent identity with
GGGCCTGGCTCGAGCAGGGGCGAGGGATT-
CGCTTCTTCCTGCCATAGCGTGG TCCCCTC-
CCCTATGGCAGGCAGAAAGCGGCACCTTCCCTC-
CCAATGACCGCGTCTTCGT CG (SEQ ID NO: 3). In a preferred embodiment, the at least one genetic element includes
CATCTCCATGGCTGTACCACCTT-
GTCGGGGGATGTGTACTTCTGAACTTGTGTTGAAT
CTCATGGAGTTCAGAAACACATCCGCACT-
GACATTTTGGTATCTTTTCATCTGACCA (SEQ ID NO:
2); or GGGCCTGGCTCGAGCAGGGGCGAGGGAT-
TCCGCTTCTTC CTGCCATAGCGTGGTCCCCTC-
CCCTATGGCAGGCAGAAAGCGGCACCTTCCCTC-
CCAAT GACCGCGTCTTCGTCTG (SEQ ID NO: 3).

In another aspect, the microRNA cluster includes a sequence having at least 80%, or at least 85%, or at least 90%, or at least 95% percent identity with AGGTATATT-
GCTGTTGACAGTGAGCGACTGTAACTGAGCTT-
GCTCTACTGTGAAGCC ACAGATGGGTAGAG-
CAAGCACAGTTTACCGCTGCCTACTGCCTCGGACT
TCAAGGGG CTTCGGGGCATCTCCATGGCTGTAC-
CACCTTGTTCGGGGGATGTGTACTTCTGAACTT
GTGTTGAATCTCATGGAGTTCAGAAACACATC-
CGCACTGACATTTTGGTATCTTTC ATCTGACCA-
GCTAGCGGGCCTGGCTCGAGCAGGGGGC-
GAGGGATTCCGCTTCTTCCT
GCCATAGCGTGGTCCCCTCCCCTATGGCAGGCA-
GAAGCGGCACCTTCCCTCCCAATGA CCGCGTCT-
TCGTC (SEQ ID NO: 31). In a preferred embodiment, the microRNA cluster includes: AGGTATATTGCTGTTGACA-
GTGAGCGACTGTAACTGAGCTTGCTCT ACTGT-
GAAGCCACAGATGGGTAGAGCAAGCACAGTTTAC-
CGCTGCCTACTGCCTCGG
ACTTCAAGGGGCTTCCCGGCATCTCCATGGCTG-
TACCACCTTGTTCGGGGGATGTGTA CTCTGAACTT-
GTGTTGAATCTCATGGAGTTCAGAAACACATC-
CGCACTGACATTT
TGGTATCTTTTCATCTGACCAGCTAGCGGGCCTG-
GCTCGAGCAGGGGCGAGGGATTG CGCTTCTTC-
CTGCCATAGCGTGGTCCCCTCCCCTATGGCAGGCA-
GAAGCGGCACCTTC
CCTCCCAATGACCGCGTCTTCGTCTG (SEQ ID NO: 31).

In another aspect, a lentiviral vector is disclosed. The lentiviral vector includes at least one encoded genetic element, wherein the at least one encoded genetic element comprises a small RNA capable of inhibiting production of chemokine receptor CCR5. The at least one encoded genetic element may also comprise at least one small RNA capable of targeting an HIV RNA sequence. In another aspect, the at least one encoded genetic element comprises a small RNA capable of inhibiting production of chemokine receptor CCR5 and at least one small RNA capable of targeting an HIV RNA sequence. The HIV RNA sequence may include a HIV Vif sequence, a HIV Tat sequence, or a variant thereof. The at least one encoded genetic element may

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include a microRNA or a shRNA. The at least one encoded genetic element may include a microRNA cluster.

In another aspect, the at least one genetic element includes a microRNA having at least 80%, or at least 85%, or at least 90%, or at least 95% percent identity with AGGTATATT-
GCTGTTGACAGTGAGCGACTGTAACTGAGCTT-
GCTCTACTGTGAAGCC ACAGATGGGTAGAG-
CAAGCACAGTTTACCGCTGCCTACTGCCTCGGACT
TCAAGGGG CTT (SEQ ID NO: 1). In a preferred embodi-
ment, the at least one genetic element comprises: AGGTAT-
ATTGCTGTTGACAGTGAGCGACTGTAACTGAGCT-
TGCTCTACTGTGAAGCC
ACAGATGGGTAGAGCAAGCACAGTTTACCGCTGC-
CTACTGCCTCGGACTTCAAGGGG CTT (SEQ ID NO:
1).

In another aspect, the at least one genetic element includes a microRNA having at least 80%, or at least 85%, or at least 90%, or at least 95% percent identity with CATCTCCATG-
GCTGTACCACCTTGTTCGGGGGATGTGTACTTCT-
GAACTTGTGTTGAAT CTCATGGAGTTCA-
GAAGAACACATCCGCACTGACATTTTGGTATCTTTC
ATCTGACCA (SEQ ID NO: 2); or at least 80%, or at least 85%, or at least 90%, or at least 95% percent identity with
GGGCCTGGCTCGAGCAGGGGCGAGGGATT-
CGCTTCTTCCTGCCATAGCGTGG TCCCCTC-
CCCTATGGCAGGCAGAAAGCGGCACCTTCCCTC-
CCAATGACCGCGTCTTCGT CG (SEQ ID NO: 3). In a preferred embodiment, the at least one genetic element includes
CATCTCCATGGCTGTACCACCTT-
GTCGGGGGATGTGTACTTCTGAACTTGTGTTGAAT
CTCATGGAGTTCAGAAACACATCCGCACT-
GACATTTTGGTATCTTTTCATCTGACCA (SEQ ID NO:
2); or GGGCCTGGCTCGAGCAGGGGCGAGGGAT-
TCCGCTTCTTC CTGCCATAGCGTGGTCCCCTC-
CCCTATGGCAGGCAGAAAGCGGCACCTTCCCTC-
CCAAT GACCGCGTCTTCGTCTG (SEQ ID NO: 3).

In another aspect, the microRNA cluster includes a sequence having at least 80%, or at least 85%, or at least 90%, or at least 95% percent identity with AGGTATATT-
GCTGTTGACAGTGAGCGACTGTAACTGAGCTT-
GCTCTACTGTGAAGCC ACAGATGGGTAGAG-
CAAGCACAGTTTACCGCTGCCTACTGCCTCGGACT
TCAAGGGG CTTCGGGGCATCTCCATGGCTGTAC-
CACCTTGTTCGGGGGATGTGTACTTCTGAACTT
GTGTTGAATCTCATGGAGTTCAGAAACACATC-
CGCACTGACATTTTGGTATCTTTC ATCTGACCA-
GCTAGCGGGCCTGGCTCGAGCAGGGGGC-
GAGGGATTCCGCTTCTTCCT
GCCATAGCGTGGTCCCCTCCCCTATGGCAGGCA-
GAAGCGGCACCTTCCCTCCCAATGA CCGCGTCT-
TCGTC (SEQ ID NO: 31). In a preferred embodiment, the microRNA cluster includes: AGGTATATTGCTGTTGACA-
GTGAGCGACTGTAACTGAGCTTGCTCT ACTGT-
GAAGCCACAGATGGGTAGAGCAAGCACAGTTTAC-
CGCTGCCTACTGCCTCGG
ACTTCAAGGGGCTTCCCGGCATCTCCATGGCTG-
TACCACCTTGTTCGGGGGATGTGTA CTCTGAACTT-
GTGTTGAATCTCATGGAGTTCAGAAACACATC-
CGCACTGACATTT
TGGTATCTTTTCATCTGACCAGCTAGCGGGCCTG-
GCTCGAGCAGGGGCGAGGGATTG CGCTTCTTC-
CTGCCATAGCGTGGTCCCCTCCCCTATGGCAGGCA-
GAAGCGGCACCTTC
CCTCCCAATGACCGCGTCTTCGTCTG (SEQ ID NO: 31).

In another aspect, a lentiviral vector system for expressing a lentiviral particle is disclosed. The system includes a lentiviral vector as described herein; an envelope plasmid

for expressing an envelope protein preferably optimized for infecting a cell; and at least one helper plasmid for expressing genes of interest. In embodiments, the genes of interest include one or more of gag, pol, and rev genes. In embodiments, the lentiviral vector, the envelope plasmid, and the at least one helper plasmid are transfected into a packaging cell line. In further embodiments, a lentiviral particle is produced by the packaging cell line. In embodiments, the lentiviral particle is capable of modulating production of a target of interest. In embodiments, the target of interest is any of chemokine receptor CCR5 or an HIV RNA sequence. The system may further include a first helper plasmid and a second helper plasmid. In embodiments, a first helper plasmid expresses the gag and pol genes, and a second helper plasmid expresses the rev gene.

In another aspect, a lentiviral particle capable of infecting a cell is provided. The lentiviral particle includes an envelope protein preferably optimized for infecting a cell, and a lentiviral vector as described herein. In embodiments, the envelope protein may be optimized for infecting a T cell. In a preferred embodiment, the envelope protein is optimized for infecting a CD4+ T cell.

In another aspect, a modified cell is provided. The modified cell includes any cell capable of being infected with a lentiviral vector system for use in accordance with present aspects and embodiments. In embodiments, the cell is a CD4+ T cell that is infected with a lentiviral particle. In embodiments, the CD4+ T cell also has been selected to recognize an HIV antigen. In embodiments, the HIV antigen includes a gag antigen. In embodiments, the CD4+ T cell expresses a decreased level of CCR5 following infection with the lentiviral particle.

In another aspect, a method of selecting a subject for a therapeutic treatment regimen is provided. The method variously includes immunizing the subject with an effective amount of a first stimulatory agent; removing leukocytes from the subject and purifying peripheral blood mononuclear cells (PBMC) and determining a first quantifiable measurement associated with at least one factor associated with the PBMC; contacting the PBMC ex vivo with a therapeutically effective amount of a second stimulatory agent, and determining a second measurement associated with the at least one factor associated with the PBMC, whereby when the second quantifiable measurement is higher than the first quantifiable measurement, the subject is selected for the treatment regimen. The at least one factor may include any of T cell proliferation or IFN gamma production.

The foregoing general description and following brief description of the drawings and detailed description are exemplary and explanatory and are intended to provide further explanation of the invention as claimed. Other objects, advantages, and novel features will be readily apparent to those skilled in the art from the following brief description of the drawings and detailed description of the invention.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 depicts a flow diagram of an ex vivo treatment method of the present disclosure.

FIG. 2 depicts CD4+ T cell alteration and prevention of new infection in accordance with the present disclosure.

FIG. 3 depicts an exemplary lentiviral vector system comprised of a therapeutic vector, a helper plasmid, and an envelope plasmid. The therapeutic vector shown here is a

preferred therapeutic vector, which is also referred to herein as AGT103, and contains miR30CCR5-miR21Vif-miR185-Tat.

FIGS. 4A-4C depict an exemplary 3-vector lentiviral vector system in a circularized form.

FIGS. 5A-5D depict an exemplary 4-vector lentiviral vector system in a circularized form.

FIG. 6 depicts exemplary vector sequences. Positive (i.e., genomic) strand sequences of the promoter and miR cluster were developed for inhibiting the spread of CCR5-tropic HIV strains. Sequences that are not underlined comprise the EF-1alpha promoter of transcription that was selected as being a preferable promoter for this miR cluster. Sequences that are underlined show the miR cluster consisting of miR30 CCR5, miR21 Vif, and miR185 Tat (as shown collectively in SEQ ID NO: 33).

FIG. 7 depicts exemplary lentiviral vector constructs according to various aspects of this disclosure.

FIGS. 8A-8B show knockdown of CCR5 by an experimental vector and corresponding prevention of R5-tropic HIV infection in AGTc120 cells. FIG. 8A shows CCR5 expression in AGTc120 cells with or without AGT103 lentivirus vector. FIG. 8B shows the sensitivity of transduced AGTc120 cells to infection with a HIV BaL virus stock that was expressing green fluorescent protein (GFP) fused to the Nef gene of HIV.

FIGS. 9A-9B depict data demonstrating regulation of CCR5 expression by shRNA inhibitor sequences in a lentiviral vector of the present disclosure. FIG. 9A shows screening data for potential candidates. FIG. 9B shows CCR5 knock-down data following transduction with CCR5 shRNA-1 (SEQ ID NO: 16).

FIGS. 10A-10B depict data demonstrating regulation of HIV components by shRNA inhibitor sequences in a lentiviral vector of the present disclosure. FIG. 10A shows knock-down data for the rev/tat target gene. FIG. 10B shows knock-down data for the gag target gene.

FIG. 11 depicts data demonstrating that AGT103 reduces expression of Tat protein expression in cells transfected with an HIV expression plasmid, as described herein.

FIGS. 12A-12B depict data demonstrating regulation of HIV components by synthetic microRNA sequences in a lentiviral vector of the present disclosure. In FIG. 12A, tat knock-down data is shown. In FIG. 12B, vif knock-down data is shown.

FIG. 13 depicts data demonstrating regulation of CCR5 expression by synthetic microRNA sequences in a lentiviral vector of the present disclosure.

FIG. 14 depicts data demonstrating regulation of CCR5 expression by synthetic microRNA sequences in a lentiviral vector of the present disclosure containing either a long or short WPRE sequence.

FIG. 15 depicts data demonstrating regulation of CCR5 expression by synthetic microRNA sequences in a lentiviral vector of the present disclosure with or without a WPRE sequence.

FIG. 16 depicts data demonstrating regulation of CCR5 expression by a CD4 promoter regulating synthetic microRNA sequences in a lentiviral vector of the present disclosure.

FIG. 17 depicts data demonstrating detection of HIV Gag-specific CD4 T cells.

FIGS. 18A-18E depict data demonstrating HIV-specific CD4 T cell expansion and lentivirus transduction. FIG. 18A shows an exemplary schedule of treatment. FIG. 18B shows IFN-gamma production in CD4-gated T cells, as described herein. FIG. 18C shows IFN-gamma production and GFP

expression in CD4-gated T cells, as described herein. FIG. 18D shows frequency of HIV-specific CD4+ T cells, as described herein. FIG. 18E shows IFN-gamma production from PBMCs post-vaccination, as described herein.

FIGS. 19A-19C depict data demonstrating a functional assay for a dose response of increasing AGT103-GFP and inhibition of CCR5 expression. FIG. 19A shows dose response data for increasing amounts of AGT103-GFP. FIG. 19B shows normally distributed populations in terms of CCR5 expression. FIG. 19C shows percentage inhibition of CCR5 expression with increasing doses of AGT103-GFP.

FIGS. 20A-20B depict data demonstrating AGT103 transduction efficiency for primary human CD4+ T cells. FIG. 20A shows frequency of transduced cells (GFP-positive) by FACS, as described herein. FIG. 20B shows number of vector copies per cell, as described herein.

FIG. 21 depicts data demonstrating AGT103 inhibition of HIV replication in primary CD4+ T cells, as described herein.

FIG. 22 depicts data demonstrating AGT103 protection of primary human CD4+ T cells from HIV-induced depletion.

FIGS. 23A-D depict data demonstrating generation of a CD4+ T cell population that is highly enriched for HIV-specific, AGT103-transduced CD4 T cells. FIG. 23A shows CD4 and CD8 expression profiles for cell populations, as described herein. FIG. 23B shows CD4 and CD8 expression profiles for cell populations, as described herein. FIG. 23C shows IFN-gamma and CD4 expression profiles for cell populations, as described herein. FIG. 23D shows IFN-gamma and GFP expression profiles for cell populations, as described herein.

DETAILED DESCRIPTION

Overview

Disclosed herein are methods and compositions for treating and/or preventing human immunodeficiency virus (HIV) disease to achieve a functional cure. The methods and compositions include integrating lentivirus, non-integrating lentivirus, and related viral vector technology as described below.

Disclosed herein are therapeutic viral vectors (e.g., lentiviral vectors), immunotherapies, and methods for their use for treating HIV infection. In embodiments, methods and compositions for achieving a functional cure for HIV infection are provided. As depicted in FIG. 1 herein, the various aspects and embodiments include a first stimulation event, for example a first therapeutic immunization with vaccines intended to produce strong immune responses against HIV in HIV-infected patients, for example with stable suppression of viremia due to daily administration of HAART. In embodiments, the first stimulation event enriches the fraction of HIV-specific CD4 T cells. This is followed by (1) isolating peripheral leukocytes by leukapheresis or purifying PBMC from venous blood, (2) a second stimulating event, for example re-stimulating CD4 T cells ex vivo with a suitable stimulatory agent, such as any vaccine or protein, for example, HIV or HIV-related peptides, (3) performing therapeutic lentivirus transduction, ex vivo T cell culture, and (4) re-infusion back into the original patient.

The various methods and compositions can be used to prevent new cells, such as CD4+ T cells, from becoming infected with HIV. For example as illustrated in FIG. 2, to prevent new cells from becoming infected, CCR5 expression can be targeted to prevent virus attachment. Further, destruction of any residual infecting viral RNA can also be targeted. In respect of the foregoing, and in reference to FIG.

2 herein, compositions and methods are provided to stop the HIV viral cycle in cells that have already become infected with HIV. To stop the HIV viral cycle, viral RNA produced by latently-infected cells, such as latently-infected CD4+ T cells, is targeted.

Previous efforts to achieve a cure for HIV have fallen short due to, among others, the failure to obtain sufficient numbers of HIV-specific CD4 T cells with protective genetic modifications. When this number is below a critical threshold, a functional cure as described herein is not achieved. For example, upon termination of antiretroviral therapy HIV re-emergence generally follows. Thereafter, patients often experience rapid destruction of HIV-specific CD4 T cells, and also followed by return to progression of disease despite prior genetic therapy. By employing therapeutic immunization in accordance with the compositions and methods described herein, a new HIV treatment regimen has been developed including, in various embodiments, a functional cure.

Definitions and Interpretation

Unless otherwise defined herein, scientific and technical terms used in connection with the present disclosure shall have the meanings that are commonly understood by those of ordinary skill in the art. Further, unless otherwise required by context, singular terms shall include pluralities and plural terms shall include the singular. Generally, nomenclature used in connection with, and techniques of, cell and tissue culture, molecular biology, immunology, microbiology, genetics and protein and nucleic acid chemistry and hybridization described herein are those well-known and commonly used in the art. The methods and techniques of the present disclosure are generally performed according to conventional methods well-known in the art and as described in various general and more specific references that are cited and discussed throughout the present specification unless otherwise indicated. See, e.g.: Sambrook J. & Russell D. *Molecular Cloning: A Laboratory Manual*, 3rd ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (2000); Ausubel et al., *Short Protocols in Molecular Biology: A Compendium of Methods from Current Protocols in Molecular Biology*, Wiley, John & Sons, Inc. (2002); Harlow and Lane *Using Antibodies: A Laboratory Manual*; Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1998); and Coligan et al., *Short Protocols in Protein Science*, Wiley, John & Sons, Inc. (2003). Any enzymatic reactions or purification techniques are performed according to manufacturer's specifications, as commonly accomplished in the art or as described herein. The nomenclature used in connection with, and the laboratory procedures and techniques of, analytical chemistry, synthetic organic chemistry, and medicinal and pharmaceutical chemistry described herein are those well-known and commonly used in the art.

As used herein, the term "about" will be understood by persons of ordinary skill in the art and will vary to some extent depending upon the context in which it is used. If there are uses of the term which are not clear to persons of ordinary skill in the art given the context in which it is used, "about" will mean up to plus or minus 10% of the particular term.

As used herein, the terms "administration of" or "administering" an active agent means providing an active agent of the invention to the subject in need of treatment in a form

that can be introduced into that individual's body in a therapeutically useful form and therapeutically effective amount.

As used herein, the term "AGT103" refers to a particular embodiment of a lentiviral vector that contains a miR30-CCR5/miR21-Vif/miR185-Tat microRNA cluster sequence, as detailed herein.

As used herein, the term "AGT103T" refers to a cell that has been transduced with a lentivirus that contains the AGT103 lentiviral vector.

Throughout this specification and claims, the word "comprise," or variations such as "comprises" or "comprising," will be understood to imply the inclusion of a stated integer or group of integers but not the exclusion of any other integer or group of integers. Further, as used herein, the term "includes" means includes without limitation.

As used herein, the term "engraftment" refers to the ability for one skilled in the art to determine a quantitative level of sustained engraftment in a subject following infusion of a cellular source (see for e.g.: Rosenberg et al., *N. Engl. J. Med.* 323:570-578 (1990); Dudley et al., *J. Immunother.* 24:363-373 (2001); Yee et al., *Curr. Opin. Immunol.* 13:141-146 (2001); Rooney et al., *Blood* 92:1549-1555 (1998)).

The terms, "expression," "expressed," or "encodes" refer to the process by which polynucleotides are transcribed into mRNA and/or the process by which the transcribed mRNA is subsequently being translated into peptides, polypeptides, or proteins. Expression may include splicing of the mRNA in a eukaryotic cell or other forms of post-transcriptional modification or post-translational modification.

The term "functional cure", as referenced above, and further defined herein, refers to a state or condition wherein HIV+ individuals who previously required ongoing HIV therapies such as cART or HAART, may survive with low or undetectable virus replication using lower doses, intermittent doses, or discontinued dosing of such HIV therapies. An individual may be said to have been "functionally cured" while still requiring adjunct therapy to maintain low level

virus replication and slow or eliminate disease progression. A possible outcome of a functional cure is the eventual eradication of all or virtually all HIV such that no recurrence is detected within a specified time frame, for example, 1 month, 3 months, 6 months, 1 year, 3 years, and 5 years, and all other time frames as may be defined.

The term "HIV vaccine" encompasses immunogens plus vehicle plus adjuvant intended to elicit HIV-specific immune responses. The term "HIV vaccine" is within the meaning of the term "stimulatory agent" as described herein. A "HIV vaccine" may include purified or whole inactivated virus particles that may be HIV or a recombinant virus vectors capable of expressing HIV proteins, protein fragments or peptides, glycoprotein fragments or glycopeptides, in addition to recombinant bacterial vectors, plasmid DNA or RNA capable of directing cells to producing HIV proteins, glycoproteins or protein fragments able to elicit specific immunity. Alternately, specific methods for immune stimulation including anti-CD3/CD28 beads, T cell receptor-specific antibodies, mitogens, superantigens and other chemical or biological stimuli may be used to activate dendritic, T or B cells for the purposes of enriching HIV-specific CD4 T cells prior to transduction or for in vitro assay of lentivirus-transduced CD4 T cells. Activating substances may be soluble, polymeric assemblies, liposome or endosome-based or linked to beads. Cytokines including interleukin-2, 6, 7, 12, 15, 23 or others may be added to improve cellular responses to stimuli and/or improve the survival of CD4 T cells throughout the culture and transduction intervals. Alternately, and without limiting any of the foregoing, the term "HIV vaccine" encompasses the MVA/HIV62B vaccine and variants thereof. The MVA/HIV62B vaccine is a known highly attenuated double recombinant MVA vaccine. The MVA/HIV62B vaccine was constructed through the insertion of HIV-1 gag-pol and env sequences into the known MVA vector (see: for e.g.: Goepfert et al. (2014) *J. Infect. Dis.* 210(1): 99-110, and see WO2006026667, both of which are incorporated herein by reference). The term "HIV vaccine" also includes any one or more vaccines provided in Table 1, below.

TABLE 1

LAVI Clinical Trial ID*	Prime**
HVTN 704 AMP	VRC-HIVMAB060-00-AB
VAC89220HPX2004	Ad26.Mos.HIV Trivalent
01-I-0079	VRC4302
04/400-003-04	APL 400-003 GENEVAX-HIV
10-1074	10-1074
87 I-114	gp160 Vaccine (Immuno-AG)
96-I-0050	APL 400-003 GENEVAX-HIV
ACTG 326; PACTG 326	ALVAC vCP1452
Ad26.ENVA.01	Ad26.EnvA-01
Ad26.ENVA.01 Mucosal/IPCVD003	Ad26.EnvA-01
Ad5HVR48.ENVA.01	Ad5HVR48.ENVA.01
ANRS VAC 01	ALVAC vCP125
ANRS VAC 02	rgp 160 + peptide V3 ANRS VAC 02
ANRS VAC 03	ALVAC-HIV MN120TMG strain (vCP205)
ANRS VAC 04	LIPO-6
ANRS VAC 04 bis	LIPO-6
ANRS VAC 05	ALVAC vCP125
ANRS VAC 06	ALVAC vCP125
ANRS VAC 07	ALVAC vCP300
ANRS VAC 08	ALVAC-HIV MN120TMG strain (vCP205)
ANRS VAC 09	ALVAC-HIV MN120TMG strain (vCP205)
ANRS VAC 09 bis	LIPO-6
ANRS VAC 10	ALVAC vCP1452
ANRS VAC 12	LPHIV1
ANRS VAC 14	gp160 MN/LAI
ANRS VAC 16	LPHIV1
ANRS VAC 17	LIPO-6
ANRS VAC 18	LIPO-5

TABLE 1-continued

IAVI Clinical Trial ID*	Prime**
APL 400-003RX101	APL 400-003 GENEVAX-HIV
AVEG 002	HIVAC-1e
AVEG 002A	HIVAC-1e
AVEG 002B	HIVAC-1e
AVEG 003	VaxSyn gp160 Vaccine (MicroGeneSys)
AVEG 003A	VaxSyn gp160 Vaccine (MicroGeneSys)
AVEG 003B	VaxSyn gp160 Vaccine (MicroGeneSys)
AVEG 004	gp160 Vaccine (Immuno-AG)
AVEG 004A	gp160 Vaccine (Immuno-AG)
AVEG 004B	gp160 Vaccine (Immuno-AG)
AVEG 005A/B	Env 2-3
AVEG 005C	Env 2-3
AVEG 006X; VEU 006	MN rgp120
AVEG 007A/B	rgp120/HIV-1 SF-2
AVEG 007C	rgp120/HIV-1 SF-2
AVEG 008	HIVAC-1e
AVEG 009	MN rgp120
AVEG 010	HIVAC-1e
AVEG 011	UBI HIV-1 Peptide Immunogen, Multivalent
AVEG 012A/B	ALVAC vCP125
AVEG 013A	gp160 Vaccine (Immuno-AG)
AVEG 013B	gp160 Vaccine (Immuno-AG)
AVEG 014A/B	TBC-3B
AVEG 014C	TBC-3B
AVEG 015	rgp120/HIV-1 SF-2
AVEG 016	MN rgp120
AVEG 016A	MN rgp120
AVEG 016B	MN rgp120
AVEG 017	UBI HIV-1 Peptide Vaccine, Microparticulate Monovalent
AVEG 018	UBI HIV-1 Peptide Vaccine, Microparticulate Monovalent
AVEG 019	p17/p24:Ty-VLP
AVEG 020	gp120 C4-V3
AVEG 021	P3C541b Lipopeptide
AVEG 022	ALVAC-HIV MN120TMG strain (vCP205)
AVEG 022A	ALVAC-HIV MN120TMG strain (vCP205)
AVEG 023	UBI HIV-1 Peptide Immunogen, Multivalent
AVEG 024	rgp120/HIV-1 SF-2
AVEG 026	ALVAC vCP300
AVEG 027	ALVAC-HIV MN120TMG strain (vCP205)
AVEG 028	Salmonella typhi CVD 908-HIV-1 LAI gp 120
AVEG 029	ALVAC-HIV MN120TMG strain (vCP205)
AVEG 031	APL 400-047
AVEG 032	ALVAC-HIV MN120TMG strain (vCP205)
AVEG 033	ALVAC-HIV MN120TMG strain (vCP205)
AVEG 034/034A	ALVAC vCP1433
AVEG 036	MN rgp120
AVEG 038	ALVAC-HIV MN120TMG strain (vCP205)
AVEG 201	rgp120/HIV-1 SF-2
AVEG 202/HIVNET 014	ALVAC-HIV MN120TMG strain (vCP205)
C060301	GTU-MultiHIV
C86P1	HIV gp140 ZM96
Cervico-vaginal CN54gp140-hsp70 Conjugate Vaccine (TL01)	CN54gp140
CM235 and SF2gp120	CM235 (ThaiE) gp120 plus SF2(B) gp120
CM235gp120 and SF2gp120	CM235 (ThaiE) gp120 plus SF2(B) gp120
CombiHIVvac (KombiVICHvak)	CombiHIVvac
CRC282	P2G12
CRO2049/CUT*HIVAC001	GTU-MultiHIV
CUTHIVAC002	DNA-C CN54ENV
DCVax-001	DCVax-001
DNA-4	DNA-4
DP67001	DP67001 DNA
DVP-1	EnvDNA
EN41-UGR7C	EN41-UGR7C
EnvDNA	EnvDNA
EnvPro	EnvPro
EuroNeut41	EN41-FPA2
EV01	NYVAC-C
EV02 (EuroVacc 02)	DNA-C
EV03/ANRSVAC20	DNA-C
Extention HVTN 073E/SAAVI 102	Sub C gp140
F4/AS01	F4/AS01
FIT Biotech	GTU-Nef
Guangxi CDC DNA vaccine	Chinese DNA
HGP-30 memory responses	HGP-30
HIV-CORE002	ChAdV63.HIVconsv

TABLE 1-continued

IAVI Clinical Trial ID*	Prime**
HIV-POL-001	MVA-mBN32
HIVIS 01	HIVIS-DNA
HIVIS 02	MVA-CMDR
HIVIS 03	HIVIS-DNA
HIVIS 05	HIVIS-DNA
HIVIS06	HIVIS-DNA
HIVIS07	HIVIS-DNA
HIVNET 007	ALVAC-HIV MN120TMG strain (vCP205)
HIVNET 026	ALVAC vCP1452
HPTN 027	ALVAC-HIV vCP1521
HVRF-380-131004	Vichrepol
HVTN 039	ALVAC vCP1452
HVTN 040	AVX101
HVTN 041	rgp120w61d
HVTN 042/ANRS VAC 19	ALVAC vCP1452
HVTN 044	VRC-HIVDNA009-00-VP
HVTN 045	pGA2/JS7 DNA
HVTN 048	EP HIV-1090
HVTN 049	Gag and Env DNA/PLG microparticles
HVTN 050/Merck 018	MRKAd5 HIV-1 gag
HVTN 052	VRC-HIVDNA009-00-VP
HVTN 054	VRC-HIVADV014-00-VP
HVTN 055	TBC-M335
HVTN 056	MEP
HVTN 057	VRC-HIVDNA009-00-VP
HVTN 059	AVX101
HVTN 060	HIV-1 gag DNA
HVTN 063	HIV-1 gag DNA
HVTN 064	EP HIV-1043
HVTN 065	pGA2/JS7 DNA
HVTN 067	EP-1233
HVTN 068	VRC-HIVADV014-00-VP
HVTN 069	VRC-HIVDNA009-00-VP
HVTN 070	PENNVAX-B
HVTN 071	MRKAd5 HIV-1 gag
HVTN 072	VRC-HIVDNA044-00-VP
HVTN 073	SAAVI DNA-C2
HVTN 076	VRC-HIVDNA016-00-VP
HVTN 077	VRC-HIVADV027-00-VP
HVTN 078	NYVAC-B
HVTN 080	PENNVAX-B
HVTN 082	VRC-HIVDNA016-00-VP
HVTN 083	VRC-HIVADV038-00-VP
HVTN 084	VRC-HIVADV054-00-VP
HVTN 085	VRC-HIVADV014-00-VP
HVTN 086, SAAVI 103	SAAVI MVA-C
HVTN 087	HIV-MAG
HVTN 088	Oligomeric gp140/MF59
HVTN 090	VSV-Indiana HIV gag vaccine
HVTN 092	DNA-HIV-PT123
HVTN 094	GEO-D03
HVTN 096	DNA-HIV-PT123
HVTN 097	ALVAC-HIV vCP1521
HVTN 098	PENNVAX-GP
HVTN 100	ALVAC-HIV-C (vCP2438)
HVTN 101	DNA-HIV-PT123
HVTN 102	DNA-HIV-PT123
HVTN 104	VRC-HIVMAB060-00-AB
HVTN 105	AIDSVAX B/E
HVTN 106	DNA Nat-B env
HVTN 110	Ad4-mgag
HVTN 112	HIV-1 nef/tat/vif, env pDNA vaccine
HVTN 114; GOVX-B11	AIDSVAX B/E
HVTN 116	VRC-HIVMAB060-00-AB
HVTN 203	ALVAC vCP1452
HVTN 204	VRC-HIVDNA016-00-VP
HVTN 205	pGA2/JS7 DNA
HVTN 502/Merck 023 (Step Study)	MRKAd5 HIV-1 gag/pol/nef
HVTN 503 (Phambili)	MRKAd5 HIV-1 gag/pol/nef
HVTN 505	VRC-HIVDNA016-00-VP
HVTN 702	ALVAC-HIV-C (vCP2438)
HVTN 703 AMP	VRC-HIVMAB060-00-AB
HVTN 908	pGA2/JS7 DNA
IAVI 001	DNA.HIVA
IAVI 002	DNA.HIVA
IAVI 003	MVA.HIVA
IAVI 004	MVA.HIVA
IAVI 005	DNA.HIVA

TABLE 1-continued

IAVI Clinical Trial ID*	Prime**
IAVI 006	DNA.HIVA
IAVI 008	MVA.HIVA
IAVI 009	DNA.HIVA
IAVI 010	DNA.HIVA
IAVI 011	MVA.HIVA
IAVI 016	MVA.HIVA
IAVI A001	tg.AAC09
IAVI A002	tg.AAC09
IAVI A003	AAV1-PG9
IAVI B001	Ad35-GRIN/ENV
IAVI B002	Adjuvanted GSK investigational HIV vaccine formulation 1
IAVI B003	Ad26.EnvA-01
IAVI B004	HIV-MAG
IAVI C001	ADVAX
IAVI C002	ADMVA
IAVI C003	ADMVA
IAVI C004/DHO-614	ADVAX
IAVI D001	TBC-M4
IAVI N004 HIV-CORE 004	Ad35-GRIN
IAVI P001	ADVAX
IAVI P002	ADVAX
IAVI R001	rcAd26.MOS1.HIVEnv
IAVI S001	SeV-G
IAVI V001	VRC-HIVDNA016-00-VP
IAVI V002	VRC-HIVDNA016-00-VP
IDEA EV06	DNA-HIV-PT123
IHV01	Full-Length Single Chain (FLSC)
IMPAACT P1112	VRC-HIVMAB060-00-AB
IPCAVD006	MVA mosaic
IPCAVD008	Trimeric gp140
IPCAVD009	Ad26.Mos.HIV Trivalent
IPCAVD010	Ad26.Mos.HIV Trivalent
ISS P-001	Tat vaccine
ISS P-002	Tat vaccine
LFn-p24 vaccine	LFn-p24
MCA-0835	3BNC117
Merck V520-007	Ad-5 HIV-1 gag (Merck)
MRC V001	rgp120w61d
MRK Ad5	Ad-5 HIV-1 gag (Merck)
MRKAd5 + ALVAC	MRKAd5 HIV-1 gag
Mucovac2	CN54gp140
MV1-F4	Measles Vector-GSK
MYM-V101	Virosome-Gp41
NCHECR-AE1	pHIS-HIV-AE
PACTG 230	AIDSVAX B/E
PAVE100	VRC-HIVDNA016-00-VP
PEACHI-04	ChAdV63.HIVconsv
PedVacc001 & PedVacc002	MVA.HIVA
PolyEnv1	PolyEnv1
PXVX-HIV-100-001	Ad4-mgag
RISVAC02	MVA-B
RisVac02 boost	MVA-B
RV 124	ALVAC-HIV MN120TMG strain (vCP205)
RV 132	ALVAC-HIV vCP1521
RV 135	ALVAC-HIV vCP1521
RV 138; B011	ALVAC-HIV MN120TMG strain (vCP205)
RV 144	ALVAC-HIV vCP1521
RV 151/WRAIR 984	LFn-p24
RV 156	VRC-HIVDNA009-00-VP
RV 156A	VRC-HIVDNA009-00-VP
RV 158	MVA-CMDR
RV 172	VRC-HIVDNA016-00-VP
RV 305	ALVAC-HIV vCP1521
RV 306	ALVAC-HIV vCP1521
RV 328	AIDSVAX B/E
RV 365	MVA-CMDR
RV262	Pennvax-G
SG06RS02	HIV gp140 ZM96
TAB9	TAB9
TaMoVac II	HIVIS-DNA
TAMOVAC-01-MZ	HIVIS-DNA
Tiantan vaccinia HIV Vaccine	Chinese DNA
Tiantan vaccinia HIV Vaccine and DNA	Chinese DNA
TMB-108	Ibalizumab
UBI HIV-1 MN China	UBI HIV-1 Peptide Immunogen, Multivalent
UBI HIV-1MN octameric-Australia study	UBI HIV-1 Peptide Immunogen, Multivalent
UBI V106	UBI HIV-1 Peptide Vaccine, Microparticulate

TABLE 1-continued

IAVI Clinical Trial ID*	Prime**
UCLA MIG-001	Monovalent
UCLA MIG-003	TBC-3B
UKHVCSpoke003	ALVAC-HIV MN120TMG strain (vCP205)
V24P1	DNA-CN54ENV and ZM96GPN
V3-MAPS	HIV p24/MF59 Vaccine
V520-016	V3-MAPS
V520-027	MRKAd5 HIV-1 gag/pol/nef
V526-001 MRKAd5 and MRKAd6 HIV-1	MRKAd5 HIV-1 gag/pol/nef
Trigene Vaccines	
VAX 002	MRKAd5 HIV-1 gag/pol/nef
VAX 003	MRKAd5 HIV-1 gag/pol/nef
VAX 004	MRKAd5 HIV-1 gag/pol/nef
VRC 004 (03-I-0022)	AIDSVAX B/B
VRC 006 (04-I-0172)	AIDSVAX B/E
VRC 007 (04-I-0254)	AIDSVAX B/B
VRC 008 (05-I-0148)	VRC-HIVDNA009-00-VP
VRC 009 (05-I-0081)	VRC-HIVADV014-00-VP
VRC 010 (05-I-0140)	VRC-HIVDNA016-00-VP
VRC 011(06-I-0149)	VRC-HIVDNA016-00-VP
VRC 012 (07-I-0167)	VRC-HIVADV027-00-VP
VRC 015 (08-I-0171)	VRC-HIVADV014-00-VP
VRC 016	VRC-HIVDNA016-00-VP
VRC 602	VRC-HIVMAB060-00-AB
VRC 607	VRCHIVMAB080-00-AB
VRC01LS	VRCHIVMAB080-00-AB
VR101	MVA-B
X001	CN54gp140

*IAVI is the International AIDS Vaccine Initiative, whose clinical trials database is publicly available at <http://www.iavi.org/trials-database/trials>.

**As used herein, the term "Prime" refers to the composition initially used as an immunological inoculant in a given clinical trial as referenced in Table 1 herein.

The term "in vivo" refers to processes that occur in a living organism. The term "ex vivo" refers to processes that occur outside of a living organism. For example, in vivo treatment refers to treatment that occurs within a patient's body, while ex vivo treatment is one that occurs outside of a patient's body, but still uses or accesses or interacts with tissues from that patient. Thereafter, an ex vivo treatment step may include a subsequent in vivo treatment step.

The term "miRNA" refers to a microRNA, and also may be referred to herein as "miR". The term "microRNA cluster" refers to at least two microRNAs that are situated on a vector in close proximity to each other and are co-expressed.

The term "packaging cell line" refers to any cell line that can be used to express a lentiviral particle.

The term "percent identity," in the context of two or more nucleic acid or polypeptide sequences, refer to two or more sequences or subsequences that have a specified percentage of nucleotides or amino acid residues that are the same, when compared and aligned for maximum correspondence, as measured using one of the sequence comparison algorithms described below (e.g., BLASTP and BLASTN or other algorithms available to persons of ordinary skill in the art) or by visual inspection. Depending on the application, the "percent identity" can exist over a region of the sequence being compared, e.g., over a functional domain, or, alternatively, exist over the full length of the two sequences to be compared. For sequence comparison, typically one sequence acts as a reference sequence to which test sequences are compared. When using a sequence comparison algorithm, test and reference sequences are input into a computer, subsequence coordinates are designated, if necessary, and sequence algorithm program parameters are designated. The sequence comparison algorithm then calculates the percent sequence identity for the test sequence(s) relative to the reference sequence, based on the designated program parameters.

Optimal alignment of sequences for comparison can be conducted, e.g., by the local homology algorithm of Smith & Waterman, *Adv. Appl. Math.* 2:482 (1981), by the homology alignment algorithm of Needleman & Wunsch, *J. Mol. Biol.* 48:443 (1970), by the search for similarity method of Pearson & Lipman, *Proc. Nat'l. Acad. Sci. USA* 85:2444 (1988), by computerized implementations of these algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group, 575 Science Dr., Madison, Wis.), or by visual inspection (see generally Ausubel et al., *infra*).

One example of an algorithm that is suitable for determining percent sequence identity and sequence similarity is the BLAST algorithm, which is described in Altschul et al., *J. Mol. Biol.* 215:403-410 (1990). Software for performing BLAST analyses is publicly available through the National Center for Biotechnology Information website.

The percent identity between two nucleotide sequences can be determined using the GAP program in the GCG software package (available at <http://www.gcg.com>), using a NWSgapdna.CMP matrix and a gap weight of 40, 50, 60, 70, or 80 and a length weight of 1, 2, 3, 4, 5, or 6. The percent identity between two nucleotide or amino acid sequences can also be determined using the algorithm of E. Meyers and W. Miller (CABIOS, 4:11-17 (1989)) which has been incorporated into the ALIGN program (version 2.0), using a PAM120 weight residue table, a gap length penalty of 12 and a gap penalty of 4. In addition, the percent identity between two amino acid sequences can be determined using the Needleman and Wunsch (*J. Mol. Biol.* (48):444-453 (1970)) algorithm which has been incorporated into the GAP program in the GCG software package (available at <http://www.gcg.com>), using either a Blossum 62 matrix or a PAM250 matrix, and a gap weight of 16, 14, 12, 10, 8, 6, or 4 and a length weight of 1, 2, 3, 4, 5, or 6.

The nucleic acid and protein sequences of the present disclosure can further be used as a "query sequence" to perform a search against public databases to, for example, identify related sequences. Such searches can be performed using the NBLAST and XBLAST programs (version 2.0) of Altschul, et al. (1990) *J. Mol. Biol.* 215:403-10. BLAST nucleotide searches can be performed with the NBLAST program, score=100, word length=12 to obtain nucleotide sequences homologous to the nucleic acid molecules of the invention. BLAST protein searches can be performed with the XBLAST program, score=50, wordlength=3 to obtain amino acid sequences homologous to the protein molecules of the invention. To obtain gapped alignments for comparison purposes, Gapped BLAST can be utilized as described in Altschul et al. (1997) *Nucleic Acids Res.* 25(17):3389-3402. When utilizing BLAST and Gapped BLAST programs, the default parameters of the respective programs (e.g., XBLAST and NBLAST) can be used. See <http://www.ncbi.nlm.nih.gov>.

As used herein, "pharmaceutically acceptable" refers to those compounds, materials, compositions, and/or dosage forms which are, within the scope of sound medical judgment, suitable for use in contact with the tissues, organs, and/or bodily fluids of human beings and animals without excessive toxicity, irritation, allergic response, or other problems or complications commensurate with a reasonable benefit/risk ratio.

As used herein, a "pharmaceutically acceptable carrier" refers to, and includes, any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like that are physiologically compatible. The compositions can include a pharmaceutically acceptable salt, e.g., an acid addition salt or a base addition salt (see, e.g., Berge et al. (1977) *J Pharm Sci* 66:1-19).

As used herein, the term "SEQ ID NO" is synonymous with the term "Sequence ID No."

As used herein, "small RNA" refers to non-coding RNA that are generally less than about 200 nucleotides or less in length and possess a silencing or interference function. In other embodiments, the small RNA is about 175 nucleotides or less, about 150 nucleotides or less, about 125 nucleotides or less, about 100 nucleotides or less, or about 75 nucleotides or less in length. Such RNAs include microRNA (miRNA), small interfering RNA (siRNA), double stranded RNA (dsRNA), and short hairpin RNA (shRNA). "Small RNA" of the disclosure should be capable of inhibiting or knocking-down gene expression of a target gene, for example through pathways that result in the destruction of the target gene mRNA.

As used herein, the term "stimulatory agent" refers to any exogenous agent that can stimulate an immune response, and includes, without limitation, a vaccine, a HIV vaccine, and HIV or HIV-related peptides. A stimulatory agent can preferably stimulate a T cell response.

As used herein, the term "subject" includes a human patient but also includes other mammals. The terms "subject," "individual," "host," and "patient" may be used interchangeably herein.

The term "therapeutically effective amount" refers to a sufficient quantity of the active agents of the present invention, in a suitable composition, and in a suitable dosage form to treat or prevent the symptoms, progression, or onset of the complications seen in patients suffering from a given ailment, injury, disease, or condition. The therapeutically effective amount will vary depending on the state of the patient's condition or its severity, and the age, weight, etc.,

of the subject to be treated. A therapeutically effective amount can vary, depending on any of a number of factors, including, e.g., the route of administration, the condition of the subject, as well as other factors understood by those in the art.

As used herein, the term "therapeutic vector" is synonymous with a lentiviral vector such as the AGT103 vector.

The term "treatment" or "treating" generally refers to an intervention in an attempt to alter the natural course of the subject being treated, and can be performed either for prophylaxis or during the course of clinical pathology. Desirable effects include, but are not limited to, preventing occurrence or recurrence of disease, alleviating symptoms, suppressing, diminishing or inhibiting any direct or indirect pathological consequences of the disease, ameliorating or palliating the disease state, and causing remission or improved prognosis.

The term "vaccine", which is used interchangeably with the term "therapeutic vaccine" refers to an exogenous agent that can elicit an immune response in an individual and includes, without limitation, purified proteins, inactivated viruses, virally vectored proteins, bacterially vectored proteins, peptides or peptide fragments, or virus-like particles (VLPs).

Description of Aspects of the Disclosure

As detailed herein, in one aspect, a method of treating cells infected with HIV is provided. The method generally includes contacting peripheral blood mononuclear cells (PBMC) isolated from a subject infected with HIV with a therapeutically effective amount of a stimulatory agent, wherein the contacting step is carried out ex vivo; transducing the PBMC ex vivo with a viral delivery system encoding at least one genetic element; and culturing the transduced PBMC for a period of time sufficient to achieve such transduction. In embodiments, the transduced PBMC are cultured from about 1 to about 35 days. The method may further include infusing the transduced PBMC into a subject. The subject may be a human. The stimulatory agent may include a peptide or mixture of peptides, and in a preferred embodiment includes a gag peptide. The stimulatory agent may include a vaccine. The vaccine may be a HIV vaccine, and in a preferred embodiment, the HIV vaccine is a MVA/HIV62B vaccine or a variant thereof. In a preferred embodiment, the viral delivery system includes a lentiviral particle. In embodiments, the at least one genetic element may include a small RNA capable of inhibiting production of chemokine receptor CCR5. In embodiments, the at least one genetic element includes at least one small RNA capable of targeting an HIV RNA sequence. In other embodiments, the at least one genetic element includes a small RNA capable of inhibiting production of chemokine receptor CCR5 and at least one small RNA capable of targeting an HIV RNA sequence. The HIV RNA sequence may include a HIV Vif sequence, a HIV Tat sequence, or variants thereof. The at least one genetic element may include at least one of a microRNA or a shRNA. In a preferred embodiment, the at least one genetic element comprises a microRNA cluster.

In another aspect, the at least one genetic element includes a microRNA having at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95% or more percent identity with AGGTATATTGCTGTTGACAGT-GAGCGACTGTAACTGAGCTTGCTCTACTGT-GAAGCC ACAGATGGGTAGAGCAAGCACAGTTTAC-

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CGCTGCCTACTGCCTCGGACTTCAAGGGG CTT
(SEQ ID NO: 1). In a preferred embodiment, the at least one
genetic element comprises: AGGTATATTGCTGTTGACA-
GTGAGCGACTGTAAACTGAGCTTGCTCTACTGT-
GAAGCC ACAGATGGGTAGAGCAAGCACAGTTTAC-
CGCTGCCTACTGCCTCGGACTTCAAGGGG CTT
(SEQ ID NO: 1).

In another aspect, the at least one genetic element includes
a microRNA having at least 80%, at least 81%, at least 82%,
at least 83%, at least 84%, at least 85%, at least 86%, at least
87%, at least 88%, at least 89%, at least 90%, at least 91%,
at least 92%, at least 93%, at least 94%, at least 95% or more
percent identity with CATCTCCATGGCTGTACACCTT-
GTCGGGGGATGTGTACTTCTGAACTTGTGTTGAAT
CTCATGGAGTTTCTTTCATCTGACCA (SEQ ID NO:
2); or at least 80%, at least 81%, at least 82%, at least 83%,
at least 84%, at least 85%, at least 86%, at least 87%, at least
88%, at least 89%, at least 90%, at least 91%, at least 92%,
at least 93%, at least 94%, at least 95% or more percent
identity with GGGCCTGGCTCGAGCAGGGGGC-
GAGGGATTCCGCTTCTTCCCTGCCATAGCGTGG
TCCCCTCCCCTATGGCAGGCAGAAAGCGGCACCTTC-
CCTCCCAATGACCGCGTCTTCGT CG (SEQ ID NO: 3).
In a preferred embodiment, the at least one genetic element
includes CATCTCCATGGCTGTACACCTT-
GTCGGGGGATGTGTACTTCTGAACTTGTGTTGAAT
CTCATGGAGTTTCTTTCATCTGACCA (SEQ ID NO:
2); or GGGCCTGGCTCGAGCAGGGGGCAGGGAT-
TCCGCTTCTTC CTGCCATAGCGTGGTCCCCTC-
CCATATGGCAGGCAGAAAGCGGCACCTTCCCTC-
CCAAT GACCGCGTCTTCGT CG (SEQ ID NO: 3).

In another aspect, the microRNA cluster includes a
sequence having at least 80%, at least 81%, at least 82%,
at least 83%, at least 84%, at least 85%, at least 86%, at least
87%, at least 88%, at least 89%, at least 90%, at least 91%,
at least 92%, at least 93%, at least 94%, at least 95% or more
percent identity with AGGTATATTGCTGTTGACAGT-
GAGCGACTGTAAACTGAGCTTGCTCTACTGT-
GAAGCC ACAGATGGGTAGAGCAAGCACAGTTTAC-
CGCTGCCTACTGCCTCGGACTTCAAGGGG
CTTCCCGGGCATCTCCATGGCTGTACACCTT-
GTCGGGGGATGTGTACTTCTGAACTT GTGTT-
GAATCTTATGAGTTTCTTTCATGAGCAAGAACACATCCGCACT-
GACATTTTGGTATCTTTC
ATCTGACCAGCTAGCGGGCCTGGCTCGAGCA-
GGGGGCGAGGGATTCCGCTTCTTCCT
GCCATAGCGTGGTCCCCTCCCCTATGGCAGGCA-
GAAGCGGCACCTTCCCTCCCAATGA CCGCGTCT-
TCGTC (SEQ ID NO: 31). In a preferred embodiment, the
microRNA cluster includes: AGGTATATTGCTGTTGACA-
GTGAGCGACTGTAAACTGAGCTTGCTCT ACTGT-
GAAGCCACAGATGGGTAGAGCAAGCACAGTTTAC-
CGCTGCCTACTGCCTCGG
ACTTCAAGGGGCTTCCCGGGCATCTCCATGGCTG-
TACCACCTTGTGCGGGGATGTGTA CTCTGAACTT-
GTGTTGAATCTCATGGAGTTTCTTTCATGAGCAAGAACACATC-
CGCACTGACATTT
TGGTATCTTTCATCTGACCAGCTAGCGGGCCTG-
GCTCGAGCAGGGGGCGAGGGATTC CGTCTTTC-
CTGCCATAGCGTGGTCCCCTCCCCTATGGCAGGCA-
GAAGCGGCACCTTC
CCTCCCAATGACCGCGTCTTCGTG (SEQ ID NO: 31).

In another aspect, a method of treating HIV infection in a
subject is disclosed. The method generally includes immu-
nizing the subject with an effective amount of a first stimu-

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latory agent; removing leukocytes from the subject and
purifying peripheral blood mononuclear cells (PBMC). The
method further includes contacting the PBMC ex vivo with
a therapeutically effective amount of a second stimulatory
agent; transducing the PBMC ex vivo with a viral delivery
system encoding at least one genetic element; and culturing
the transduced PBMC for a period of time sufficient to
achieve transduction. The method may further include fur-
ther enrichment of the PBMC, for example, by preferably
enriching the PBMC for CD4+ T cells. In embodiments, the
transduced PBMC are cultured from about 1 to about 35
days. The method may further involve infusing the trans-
duced PBMC into a subject. The subject may be a human.
The first and second stimulatory agents may be the same or
different from each other. The at least one of the first and
second stimulatory agents may include a peptide or mixture
of peptides. In embodiments, at least one of the first and
second stimulatory agents includes a gag peptide. The at
least one of the first and second stimulatory agents may
include a vaccine. The vaccine may be a HIV vaccine, and
in a preferred embodiment, the HIV vaccine is a MVA/
HIV62B vaccine or a variant thereof. In embodiments, the
first stimulatory agent is a HIV vaccine and the second
stimulatory agent is a gag peptide.

In embodiments, the viral delivery system includes a
lentiviral particle. In embodiments, the at least one genetic
element includes a small RNA capable of inhibiting produc-
tion of chemokine receptor CCR5. In embodiments, the at
least one genetic element includes at least one small RNA
capable of targeting an HIV RNA sequence. In embodi-
ments, the at least one genetic element includes a small RNA
capable of inhibiting production of chemokine receptor
CCR5 and at least one small RNA capable of targeting an
HIV RNA sequence. The HIV RNA sequence may include
a HIV Vif sequence, a HIV Tat sequence, or variants thereof.
The at least one genetic element may include a microRNA
or a shRNA, or a cluster thereof. In a preferred embodiment,
the at least one genetic element comprises a microRNA
cluster.

In another aspect, the at least one genetic element includes
a microRNA having at least 80%, at least 81%, at least 82%,
at least 83%, at least 84%, at least 85%, at least 86%, at least
87%, at least 88%, at least 89%, at least 90%, at least 91%,
at least 92%, at least 93%, at least 94%, at least 95% or more
percent identity with AGGTATATTGCTGTTGACAGT-
GAGCGACTGTAAACTGAGCTTGCTCTACTGT-
GAAGCC ACAGATGGGTAGAGCAAGCACAGTTTAC-
CGCTGCCTACTGCCTCGGACTTCAAGGGG CTT
(SEQ ID NO: 1). In a preferred embodiment, the at least one
genetic element comprises: AGGTATATTGCTGTTGACA-
GTGAGCGACTGTAAACTGAGCTTGCTCTACTGT-
GAAGCC ACAGATGGGTAGAGCAAGCACAGTTTAC-
CGCTGCCTACTGCCTCGGACTTCAAGGGG CTT
(SEQ ID NO: 1).

In another aspect, the at least one genetic element includes
a microRNA having at least 80%, or at least 85%, or at least
90%, or at least 95% percent identity with CATCTCCATG-
GCTGTACACCTTGTGCGGGGATGTGTACTTCT-
GAACTTGTGTTGAAT CTCATGGAGTTTCA-
GAAGAACACATCCGCACTGACATTTTGGTATCTTTC
ATCTGACCA (SEQ ID NO: 2); or at least 80%, at least
81%, at least 82%, at least 83%, at least 84%, at least 85%,
at least 86%, at least 87%, at least 88%, at least 89%, at least
90%, at least 91%, at least 92%, at least 93%, at least 94%,
at least 95% or more percent identity with GGGCCTG-
GCTCGAGCAGGGGGCGAGGGATTCCGCTTCTTCCCT-
GCCATAGCGTGG TCCCCTCCCCTATGGCAGGCA-

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GAAGCGGCACCTTCCCTCCCAATGACCGCGTCTTCGT
CG (SEQ ID NO: 3). In a preferred embodiment, the at least
one genetic element includes CATCTCCATGGCTGTAC-
CACCTTGTCGGGGGATGTGTAAGTCTGAACTTGT-
GTTGAAT CTCATGGAGTTCAGAAGAACACATCCG-
CACTGACATTTTGGTATCTTTCATCTGACCA (SEQ ID
NO: 2); or GGGCCTGGCTCGAGCAGGGGGC-
GAGGGATTCCGCTTCTTC CTGCCATAGCGTGGTC-
CCCTCCCCATATGGCAGGCAGAAGCGGCACCTTC-
CCTCCCAAT GACCGCGTCTTCGTCG (SEQ ID NO: 3).

In another aspect, the microRNA cluster includes a
sequence having at least 80%, at least 81%, at least 82%, at
least 83%, at least 84%, at least 85%, at least 86%, at least
87%, at least 88%, at least 89%, at least 90%, at least 91%,
at least 92%, at least 93%, at least 94%, at least 95% or more
percent identity with AGGTATATTGCTGTTGACAGT-
GAGCGACTGTAAACTGAGCTTGCTCTACTGT-
GAAGCC ACAGATGGGTAGAGCAAGCACAGTTTAC-
CGCTGCCTACTGCCTCGGACTTCAAGGGG
CTTCCCGGGCATCTCCATGGCTGTACCACCTT-
GTCGGGGGATGTGTAAGTCTGAACTT GTGTT-
GAATCTCATGGAGTTCAGAAGAACACATCCGCACT-
GACATTTTGGTATCTTTC
ATCTGACCAGCTAGCGGGCCTGGCTCGAGCA-
GGGGGCGAGGGATTCCGCTTCTTCCT
GCCATAGCGTGGTCCCTCCCTATGGCAGGCA-
GAAGCGGCACCTTCCCTCCCAATGA CCGCTCT-
TCGTC (SEQ ID NO: 31). In a preferred embodiment, the
microRNA cluster includes: AGGTATATTGCTGTTGACA-
GTGAGCGACTGTAAACTGAGCTTGCTCT ACTGT-
GAAGCCACAGATGGGTAGAGCAAGCACAGTTTAC-
CGCTGCCTACTGCCTCGG
ACTTCAAGGGGCTTCCCGGGCATCTCCATGGCTG-
TACCACCTTGTCGGGGGATGTGTA CTCTGAACTT-
GTGTTGAATCTCATGGAGTTCAGAAGAACACATC-
CGCACTGACATTT
TGGTATCTTTCATCTGACCAGCTAGCGGGCCTG-
GCTCGAGCAGGGGGCGAGGGATT CCGTTCTTC-
CTGCCATAGCGTGGTCCCTCCCTATGGCAGGCA-
GAAGCGGCACCTTC
CCTCCCAATGACCGCGTCTTCGTC (SEQ ID NO: 31).

In another aspect, a lentiviral vector is disclosed. The
lentiviral vector includes at least one encoded genetic ele-
ment, wherein the at least one encoded genetic element
comprises a small RNA capable of inhibiting production of
chemokine receptor CCR5 or at least one small RNA
capable of targeting an HIV RNA sequence. In another
aspect a lentiviral vector is disclosed in the at least one
encoded genetic element comprises a small RNA capable of
inhibiting production of chemokine receptor CCR5 and at
least one small RNA capable of targeting an HIV RNA
sequence. The HIV RNA sequence may include a HIV Vif
sequence, a HIV Tat sequence, or a variant thereof. The at
least one encoded genetic element may include a microRNA
or a shRNA. The at least one encoded genetic element may
include a microRNA cluster.

In another aspect, the at least one genetic element includes
a microRNA having at least 80%, at least 81%, at least 82%,
at least 83%, at least 84%, at least 85%, at least 86%, at least
87%, at least 88%, at least 89%, at least 90%, at least 91%,
at least 92%, at least 93%, at least 94%, at least 95% or more
percent identity with AGGTATATTGCTGTTGACAGT-
GAGCGACTGTAAACTGAGCTTGCTCTACTGT-
GAAGCC ACAGATGGGTAGAGCAAGCACAGTTTAC-
CGCTGCCTACTGCCTCGGACTTCAAGGGG CTT
(SEQ ID NO: 1). In a preferred embodiment, the at least one
genetic element comprises: AGGTATATTGCTGTTGACA-

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GTGAGCGACTGTAAACTGAGCTTGCTCTACTGT-
GAAGCC ACAGATGGGTAGAGCAAGCACAGTTTAC-
CGCTGCCTACTGCCTCGGACTTCAAGGGG CTT
(SEQ ID NO: 1).

In another aspect, the at least one genetic element includes
a microRNA having at least 80%, at least 81%, at least 82%,
at least 83%, at least 84%, at least 85%, at least 86%, at least
87%, at least 88%, at least 89%, at least 90%, at least 91%,
at least 92%, at least 93%, at least 94%, at least 95% or more
percent identity with CATCTCCATGGCTGTACCACCTT-
GTCGGGGGATGTGTAAGTCTGAACTTGTGTTGAAT
CTCATGGAGTTCAGAAGAACACATCCGCACT-
GACATTTTGGTATCTTTCATCTGACCA (SEQ ID NO:
2); or at least 80%, at least 81%, at least 82%, at least 83%,
at least 84%, at least 85%, at least 86%, at least 87%, at least
88%, at least 89%, at least 90%, at least 91%, at least 92%,
at least 93%, at least 94%, at least 95% or more percent
identity with GGGCCTGGCTCGAGCAGGGGGC-
GAGGGATTCCGCTTCTTCCTGCCATAGCGTGG

TCCCTCCCTATGGCAGGCAGAAGCGGCACCTTC-
CCTCCCAATGACCGCGTCTTCGT CG (SEQ ID NO: 3).
In a preferred embodiment, the at least one genetic element
includes CATCTCCATGGCTGTACCACCTT-
GTCGGGGGATGTGTAAGTCTGAACTTGTGTTGAAT
CTCATGGAGTTCAGAAGAACACATCCGCACT-
GACATTTTGGTATCTTTCATCTGACCA (SEQ ID NO:
2); or GGGCCTGGCTCGAGCAGGGGGCAGGGAT-
TCCGCTTCTTC CTGCCATAGCGTGGTCCCTC-
CCCTATGGCAGGCAGAAGCGGCACCTTCCCTC-
CCAAT GACCGCGTCTTCGTCG (SEQ ID NO: 3).

In another aspect, the microRNA cluster includes a
sequence having at least 80%, at least 81%, at least 82%, at
least 83%, at least 84%, at least 85%, at least 86%, at least
87%, at least 88%, at least 89%, at least 90%, at least 91%,
at least 92%, at least 93%, at least 94%, at least 95% or more
percent identity with AGGTATATTGCTGTTGACAGT-
GAGCGACTGTAAACTGAGCTTGCTCTACTGT-
GAAGCC ACAGATGGGTAGAGCAAGCACAGTTTAC-
CGCTGCCTACTGCCTCGGACTTCAAGGGG
CTTCCCGGGCATCTCCATGGCTGTACCACCTT-
GTCGGGGGATGTGTAAGTCTGAACTT GTGTT-
GAATCTCATGGAGTTCAGAAGAACACATCCGCACT-
GACATTTTGGTATCTTTC

ATCTGACCAGCTAGCGGGCCTGGCTCGAGCA-
GGGGGCGAGGGATTCCGCTTCTTCCT
GCCATAGCGTGGTCCCTCCCTATGGCAGGCA-
GAAGCGGCACCTTCCCTCCCAATGA CCGCTCT-
TCGTC (SEQ ID NO: 31). In a preferred embodiment, the
microRNA cluster includes: AGGTATATTGCTGTTGACA-
GTGAGCGACTGTAAACTGAGCTTGCTCT ACTGT-
GAAGCCACAGATGGGTAGAGCAAGCACAGTTTAC-
CGCTGCCTACTGCCTCGG
ACTTCAAGGGGCTTCCCGGGCATCTCCATGGCTG-
TACCACCTTGTCGGGGGATGTGTA CTCTGAACTT-
GTGTTGAATCTCATGGAGTTCAGAAGAACACATC-
CGCACTGACATTT
TGGTATCTTTCATCTGACCAGCTAGCGGGCCTG-
GCTCGAGCAGGGGGCGAGGGATT CCGTTCTTC-
CTGCCATAGCGTGGTCCCTCCCTATGGCAGGCA-
GAAGCGGCACCTTC
CCTCCCAATGACCGCGTCTTCGTC (SEQ ID NO: 31).

In another aspect, a lentiviral vector system for expressing
a lentiviral particle is provided. The system includes a
lentiviral vector as described herein; at least one envelope
plasmid for expressing an envelope protein preferably opti-
mized for infecting a cell; and at least one helper plasmid for
expressing a gene of interest, for example any of gag, pol,

and rev genes, wherein when the lentiviral vector, the at least one envelope plasmid, and the at least one helper plasmid are transfected into a packaging cell, wherein a lentiviral particle is produced by the packaging cell, wherein the lentiviral particle is capable of modulating a target sequence of interest, for example inhibiting production of chemokine receptor CCR5 or targeting an HIV RNA sequence.

In another aspect, a lentiviral particle capable of infecting a cell is disclosed. The lentiviral particle includes at least one envelope protein preferably optimized for infecting a cell, and a lentiviral vector as described herein. The envelope protein may be optimized for infecting a T cell. In a preferred embodiment, the envelope protein is optimized for infecting a CD4+ T cell.

In another aspect, a modified cell is disclosed. In embodiments, the modified cell is a CD4+ T cell. In embodiments, the CD4+ T cell is infected with a lentiviral particle as described herein. In embodiments, the CD4+ T cell also has been selected to recognize an HIV antigen based on the prior immunization with a stimulatory agent. In a further preferred embodiment, the HIV antigen that is recognized by the CD4+ T cell includes a gag antigen. In a further preferred embodiment, the CD4+ T cell expresses a decreased level of CCR5 following infection with the lentiviral particle.

In another aspect, a method of selecting a subject for a therapeutic treatment regimen is disclosed. The method generally includes immunizing the subject with an effective amount of a first stimulatory agent; removing leukocytes from the subject and purifying peripheral blood mononuclear cells (PBMC) and determining a first quantifiable measurement associated with at least one factor associated with the PBMC; contacting the PBMC ex vivo with a therapeutically effective amount of a second stimulatory agent, and determining a second measurement associated with the at least one factor associated with the PBMC, whereby when the second quantifiable measurement is different (e.g., higher) than the first quantifiable measurement, the subject is selected for the treatment regimen. The at least one factor may be T cell proliferation or IFN gamma production.

Human Immunodeficiency Virus (HIV)

Human Immunodeficiency Virus, which is also commonly referred to as "HIV", is a retrovirus that causes acquired immunodeficiency syndrome (AIDS) in humans. AIDS is a condition in which progressive failure of the immune system allows life-threatening opportunistic infections and cancers to thrive. Without treatment, average survival time after infection with HIV is estimated to be 9 to 11 years, depending upon the HIV subtype. Infection with HIV occurs by the transfer of bodily fluids, including but not limited to blood, semen, vaginal fluid, pre-ejaculate, saliva, tears, lymph or cerebro-spinal fluid, or breast milk. HIV may be present in an infected individual as both free virus particles and within infected immune cells.

HIV infects vital cells in the human immune system such as helper T cells, although tropism can vary among HIV subtypes. Immune cells that may be specifically susceptible to HIV infection include but are not limited to CD4+ T cells, macrophages, and dendritic cells. HIV infection leads to low levels of CD4+ T cells through a number of mechanisms, including but not limited to apoptosis of uninfected bystander cells, direct viral killing of infected cells, and killing of infected CD4+ T cells by CD8 cytotoxic lymphocytes that recognize infected cells. When CD4+ T cell numbers decline below a critical level, cell-mediated immunity is lost, and the body becomes progressively more susceptible to opportunistic infections and cancer.

Structurally, HIV is distinct from many other retroviruses. The RNA genome consists of at least seven structural landmarks (LTR, TAR, RRE, PE, SLIP, CRS, and INS), and at least nine genes (gag, pol, env, tat, rev, nef, vif, vpr, vpu, and sometimes a tenth tev, which is a fusion of tat, env and rev), encoding 19 proteins. Three of these genes, gag, pol, and env, contain information needed to make the structural proteins for new virus particles.

HIV replicates primarily in CD4 T cells, and causes cellular destruction or dysregulation to reduce host immunity. Because HIV establishes infection as an integrated provirus and may enter a state of latency wherein virus expression in a particular cell decreases below the level for cytopathology affecting that cell or detection by the host immune system, HIV is difficult to treat and has not been eradicated even after prolonged intervals of highly active antiretroviral therapy (HAART). In the vast majority of cases, HIV infection causes fatal disease although survival may be prolonged by HAART.

A major goal in the fight against HIV is to develop strategies for curing disease. Prolonged HAART has not accomplished this goal, so investigators have turned to alternative procedures. Early efforts to improve host immunity by therapeutic immunization (using a vaccine after infection has occurred) had marginal or no impact. Likewise, treatment intensification had moderate or no impact.

Some progress has been made using genetic therapy, but positive results are sporadic and found only among rare human beings carrying defects in one or both alleles of the gene encoding CCR5 (chemokine receptor), which plays a critical role in viral penetration of host cells. However, many investigators are optimistic that genetic therapy holds the best promise for eventually achieving an HIV cure.

As disclosed herein, the methods and compositions of the invention are able to achieve a functional cure that may or may not include complete eradication of all HIV from the body. As mentioned above, a functional cure is defined as a state or condition wherein HIV+ individuals who previously required HAART, may survive with low or undetectable virus replication and using lower or intermittent doses of HAART, or are potentially able to discontinue HAART altogether. As used herein, a functional cure may still possibly require adjunct therapy to maintain low level virus replication and slow or eliminate disease progression. A possible outcome of a functional cure is the eventual eradication of HIV to prevent all possibility of recurrence.

The primary obstacles to achieving a functional cure lie in the basic biology of HIV itself. Virus infection deletes CD4 T cells that are critical for nearly all immune functions. Most importantly, HIV infection and depletion of CD4 T cells requires activation of individual cells. Activation is a specific mechanism for individual CD4 T cell clones that recognize pathogens or other molecules, using a rearranged T cell receptor.

In the case of HIV, infection activates a population of HIV-specific T cells that become infected and are consequently depleted before other T cells that are less specific for the virus, which effectively cripples the immune system's defense against the virus. The capacity for HIV-specific T cell responses is rebuilt during prolonged HAART; however, when HAART is interrupted the rebounding virus infection repeats the process and again deletes the virus-specific cells, resetting the clock on disease progression.

Clearly, a functional cure is only possible if enough HIV-specific CD4 T cells are protected to allow for a host's native immunity to confront and control HIV once HAART is interrupted. In one embodiment, the present invention

provides methods and compositions for improving the effectiveness of genetic therapy to provide a functional cure of HIV disease. In another embodiment, the present invention provides methods and compositions for enhancing host immunity against HIV to provide a functional cure. In yet another embodiment, the present invention provides methods and compositions for enriching HIV-specific CD4 T cells in a patient to achieve a functional cure.

In one embodiment of the invention, treatment results in enriching a subject's HIV-specific CD4 T cells by about 100%, about 200%, about 300%, about 400%, about 500%, about 600%, about 700%, about 800%, about 900%, or about 1000%.

Gene Therapy

Viral vectors are used to deliver genetic constructs to host cells for the purposes of disease therapy or prevention.

Genetic constructs can include, but are not limited to, functional genes or portions of genes to correct or complement existing defects, DNA sequences encoding regulatory proteins, DNA sequences encoding regulatory RNA molecules including antisense, short homology RNA, long non-coding RNA, small interfering RNA or others, and decoy sequences encoding either RNA or proteins designed to compete for critical cellular factors to alter a disease state. Gene therapy involves delivering these therapeutic genetic constructs to target cells to provide treatment or alleviation of a particular disease.

There are multiple ongoing efforts to utilize genetic therapy in the treatment of HIV disease, but thus far, the results have been poor. A small number of treatment successes were obtained in rare HIV patients carrying a spontaneous deletion of the CCR5 gene (an allele known as CCR5delta32).

Lentivirus-delivered nucleases or other mechanisms for gene deletion/modification may be used to lower the overall expression of CCR5 and/or help to lower HIV replication. At least one study has reported having success in treating the disease when lentivirus was administered in patients with a genetic background of CCR5delta32. However, this was only one example of success, and many other patients without the CCR5delta32 genotype have not been treated as successfully. Consequently, there is a substantial need to improve the performance of viral genetic therapy against HIV, both in terms of performance for the individual viral vector construct and for improved use of the vector through a strategy for achieving functional HIV cure.

For example, some existing therapies rely on zinc finger nucleases to delete a portion of CCR5 in an attempt to render cells resistant to HIV infection. However, even after optimal treatment, only 30% of T cells had been modified by the nuclease at all, and of those that were modified, only 10% of the total CD4 T cell population had been modified in a way that would prevent HIV infection. In contrast, the disclosed methods result in virtually every cell carrying a lentivirus transgene having a reduction in CCR5 expression below the level needed to allow HIV infection.

For the purposes of the disclosed methods, gene therapy can include, but is not limited to, affinity-enhanced T cell receptors, chimeric antigen receptors on CD4 T cells (or alternatively on CD8 T cells), modification of signal transduction pathways to avoid cell death cause by viral proteins, increased expression of HIV restriction elements including TREX, SAMHD1, MxA or MxB proteins, APOBEC complexes, TRIM5-alpha complexes, tetherin (BST2), and similar proteins identified as being capable of reducing HIV replication in mammalian cells.

Immunotherapy

Historically, vaccines have been a go-to weapon against deadly infectious diseases, including smallpox, polio, measles, and yellow fever. Unfortunately, there is no currently approved vaccine for HIV. The HIV virus has unique ways of evading the immune system, and the human body seems incapable of mounting an effective immune response against it. As a result, scientists do not have a clear picture of what is needed to provide protection against HIV.

However, immunotherapy may provide a solution that was previously unaddressed by conventional vaccine approaches. Immunotherapy, also called biologic therapy, is a type of treatment designed to boost the body's natural defenses to fight infections or cancer. It uses materials either made by the body or in a laboratory to improve, target, or restore immune system function.

In some embodiments of the disclosed invention, immunotherapeutic approaches may be used to enrich a population of HIV-specific CD4 T cells for the purpose of increasing the host's anti-HIV immunity. In some embodiments of the disclosed invention, integrating or non-integrating lentivirus vectors may be used to transduce a host's immune cells for the purposes of increasing the host's anti-HIV immunity. In yet another embodiment of the invention, a vaccine comprising HIV proteins including but not limited to a killed particle, a virus-like particle, HIV peptides or peptide fragments, a recombinant viral vector, a recombinant bacterial vector, a purified subunit or plasmid DNA combined with a suitable vehicle and/or biological or chemical adjuvants to increase a host's immune responses may be used to enrich the population of virus-specific T cells or antibodies, and these methods may be further enhanced through the use of HIV-targeted genetic therapy using lentivirus or other viral vector.

Methods

In one aspect, the disclosure provides methods for using viral vectors to achieve a functional cure for HIV disease. The methods generally include immunotherapy to enrich the proportion of HIV-specific CD4 T cells, followed by lentivirus transduction to deliver inhibitors of HIV and CCR5 and CXCR4 as required.

In one embodiment, the methods include a first stimulation event to enrich a proportion of HIV-specific CD4 T cells. The first stimulation can include administration of one or more of any agent suitable for enriching a patient's HIV-specific CD4+ T cells including but not limited to a vaccine.

Therapeutic vaccines can include one or more HIV protein with protein sequences representing the predominant viral types of the geographic region where treatment is occurring. Therapeutic vaccines will include purified proteins, inactivated viruses, virally vectored proteins, bacterially vectored proteins, peptides or peptide fragments, virus-like particles (VLPs), biological or chemical adjuvants including cytokines and/or chemokines, vehicles, and methods for immunization. Vaccinations may be administered according to standard methods known in the art and HIV patients may continue antiretroviral therapy during the interval of immunization and subsequent ex vivo lymphocyte culture including lentivirus transduction.

In some embodiments, HIV+ patients are immunized with an HIV vaccine, increasing the frequency of HIV-specific CD4 T cells by about 2, about 25, about 250, about 500, about 750, about 1000, about 1250, or about 1500-fold (or any amount in between these values). The vaccine may be any clinically utilized or experimental HIV vaccine, including the disclosed lentiviral, other viral vectors or other

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bacterial vectors used as vaccine delivery systems. In another embodiment, the vectors encode virus-like particles (VLPs) to induce higher titers of neutralizing antibodies. In another embodiment, the vectors encode peptides or peptide fragments associated with HIV including but not limited to gag, pol, and env, tat, rev, nef, vif, vpr, vpu, and tev, as well as LTR, TAR, RRE, PE, SLIP, CRS, and INS. Alternatively, the HIV vaccine used in the disclosed methods may comprise purified proteins, inactivated viruses, virally vectored proteins, bacterially vectored proteins, peptides or peptide fragments, virus-like particles (VLPs), or biological or chemical adjuvants including cytokines and/or chemokines.

In one embodiment, the methods include ex vivo re-stimulation of CD4 T cells from persons or patients previously immunized by therapeutic vaccination, using purified proteins, inactivated viruses, virally vectored proteins, bacterially vectored proteins, biological or chemical adjuvants including cytokines and/or chemokines, vehicles, and methods for re-stimulation. Ex vivo re-stimulation may be performed using the same vaccine or immune stimulating compound used for in vivo immunization, or it may be performed using a different vaccine or immune stimulating compound than those used for in vivo immunization. Moreover, in some embodiments, the patient does not require prior therapeutic vaccination or re-stimulation of CD4 T cells if the individual has sufficiently high antigen-specific CD4 T cell responses to HIV proteins. In these embodiments, such a patient may only require administration of the disclosed viral vectors to achieve a functional cure.

In embodiments, peripheral blood mononuclear cells (PBMCs) are obtained by leukapheresis and treated ex vivo to obtain about 1×10^{10} CD4 T cells of which about 0.1%, about 1%, about 5% or about 10% or about 30% are both HIV-specific in terms of antigen responses, and HIV-resistant by virtue of carrying the therapeutic transgene delivered by the disclosed lentivirus vector. Alternatively, about 1×10^7 , about 1×10^8 , about 1×10^9 , about 1×10^{10} , about 1×10^{11} , or about 1×10^{12} CD4 T cells may be isolated for re-stimulation. Any suitable amount of CD4 T cells are isolated for ex vivo re-stimulation.

The isolated CD4 T cells can be cultured in appropriate medium throughout re-stimulation with HIV vaccine antigens, which may include antigens present in the prior therapeutic vaccination. Antiretroviral therapeutic drugs including inhibitors of reverse transcriptase, protease or integrase may be added to prevent virus re-emergence during prolonged ex vivo culture. CD4 T cell re-stimulation is used to enrich the proportion of HIV-specific CD4 T cells in culture. The same procedure may also be used for analytical objectives wherein smaller blood volumes with peripheral blood mononuclear cells obtained by purification, are used to identify HIV-specific T cells and measure the frequency of this sub-population.

The PBMC fraction may be enriched for HIV-specific CD4 T cells by contacting the cells with HIV proteins matching or complementary to the components of the vaccine previously used for in vivo immunization. Ex vivo re-stimulation can increase the relative frequency of HIV-specific CD4 T cells by about 5, about 10, 25, about 50, about 75, about 100, about 125, about 150, about 175, or about 200-fold.

The methods additionally include combining in vivo therapeutic immunization and ex vivo re-stimulation of CD4 T cells with ex vivo lentiviral transduction and culturing.

Thus, in one embodiment, the re-stimulated PBMC fraction that has been enriched for HIV-specific CD4 T cells can be transduced with therapeutic anti-HIV lentivirus or other

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vectors and maintained in culture for a sufficient period of time for such transduction, for example from about 1 to about 21 days, including up to about 35 days. Alternatively, the cells may be cultured for about 1-about 18 days, about 1-about 15 days, about 1-about 12 days, about 1-about 9 days, or about 3-about 7 days. Thus, the transduced cells may be cultured for about 1, about 2, about 3, about 4, about 5, about 6, about 7, about 8, about 9, about 10, about 11, about 12, about 13, about 14, about 15, about 16, about 17, about 18, about 19, about 20, about 21, about 22, about 23, about 24, about 25, about 26, about 27, about 28, about 29, about 30, about 31, about 32, about 33, about 34, or about 35 days.

In further embodiments, once the transduced cells have been cultured for a sufficient period of time, transduced CD4 T cells are infused back into the original patient. Infusion can be performed using various devices and methods known in the art. In some embodiments, infusion may be accompanied by pre-treatment with cyclophosphamide or similar compounds to increase the efficiency of re-engraftment.

In some embodiments, a CCR5-targeted therapy may be added to a subject's antiretroviral therapy regimen, which was continued throughout the treatment process. Examples of CCR5-targeted therapies include but are not limited to Maraviroc (a CCR5 antagonist) or Rapamycin (immunosuppressive agent that lowers CCR5). In some embodiments, the antiretroviral therapy may be ceased and the subject can be tested for virus rebound. If no rebound occurs, adjuvant therapy can also be removed and the subject can be tested again for virus rebound.

In various embodiments, continued virus suppression with reduced or no antiretroviral therapy including cART or HAART, and reduced or no adjuvant therapy for about 26 weeks can be considered a functional cure for HIV. Other definitions of a functional cure are described herein.

The lentiviral and other vectors used in the disclosed methods may encode at least one, at least two, at least three, at least four, or at least five genes, or at least six genes, or at least seven genes, or at least eight genes, or at least nine genes, or at least ten genes, or at least eleven genes, or at least twelve genes of interest. Given the versatility and therapeutic potential of HIV-targeted gene therapy, a viral vector of the invention may encode genes or nucleic acid sequences that include but are not limited to (i) an antibody directed to an antigen associated with an infectious disease or a toxin produced by the infectious pathogen, (ii) cytokines including interleukins that are required for immune cell growth or function and may be therapeutic for immune dysregulation encountered in HIV and other chronic or acute human viral or bacterial pathogens, (iii) factors that suppress the growth of HIV in vivo including CD8 suppressor factors, (iv) mutations or deletions of chemokine receptor CCR5, mutations or deletions of chemokine receptor CXCR4, or mutations or deletions of chemokine receptor CXCR5, (v) antisense DNA or RNA against specific receptors or peptides associated with HIV or host protein associated with HIV, (vi) small interfering RNA against specific receptors or peptides associated with HIV or host protein associated with HIV, or (vii) a variety of other therapeutically useful sequences that may be used to treat HIV or AIDS.

Additional examples of HIV-targeted gene therapy that can be used in the disclosed methods include, but are not limited to, affinity-enhanced T cell receptors, chimeric antigen receptors on CD4 T cells (or alternatively on CD8 T cells), modification of signal transduction pathways to avoid cell death cause by viral proteins, increased expression of HIV restriction elements including TREX, SAMHD1, MxA

or MxB proteins, APOBEC complexes, TRIM5-alpha complexes, tetherin (BST2), and similar proteins identified as being capable of reducing HIV replication in mammalian cells.

In some embodiments, a patient may be undergoing cART or HAART concurrently while being treated according to the methods of the invention. In other embodiments, a patient may undergo cART or HAART before or after being treated according to the methods of the invention. In some embodiments, cART or HAART is maintained throughout treatment according to the methods of the invention and the patient may be monitored for HIV viral burden in blood and frequency of lentivirus-transduced CD4 T cells in blood. Preferably, a patient receiving cART or HAART prior to being treated according to the methods of the invention is able to discontinue or reduce cART or HAART following treatment according to the methods of the invention.

For efficacy purposes, the frequency of transduced, HIV-specific CD4 T cells, which is a novel surrogate marker for gene therapy effects, may be determined, as discussed in more detail herein.

Compositions

In various aspects, the disclosure provides lentiviral vectors capable of delivering genetic constructs to inhibit HIV penetration of susceptible cells. For instance, one mechanism of action in accordance herein is to reduce mRNA levels for CCR5 and/or CXCR4 chemokine receptors for reducing the rates for viral entry into susceptible cells.

Alternatively, the disclosed lentiviral vectors are capable of inhibiting the formation of HIV-infected cells by reducing the stability of incoming HIV genomic RNA. And in yet another embodiment, the disclosed lentivirus vectors are capable of preventing HIV production from a latently infected cell, wherein the mechanism of action is to cause instability of viral RNA sequences through the action of inhibitory RNA including short-homology, small-interfering or other regulatory RNA species.

The therapeutic lentiviruses disclosed generally comprise at least one of two types of genetic cargo. First, the lentiviruses may encode genetic elements that direct expression of small RNA capable of inhibiting the production of chemokine receptors CCR5 and/or CXCR4 that are important for HIV penetration of susceptible cells. The second type of genetic cargo includes constructs capable of expressing small RNA molecules targeting HIV RNA sequences for the purpose of preventing reverse transcription, RNA splicing, RNA translation to produce proteins, or packaging of viral genomic RNA for particle production and spreading infection. An exemplary structure is diagrammed in FIG. 3.

As shown in FIG. 3 (top panel), an exemplary construct may comprise numerous sections or components. For example, in one embodiment, an exemplary LV construct may comprise the following sections or components:

RSV—a Rous Sarcoma virus long terminal repeat;

5'LTR—a portion of an HIV long terminal repeat that can be truncated to prevent replication of the vector after chromosomal integration;

Psi—a packaging signal that allows for incorporation of the vector RNA genome into viral particles during packaging;

RRE—a Rev Responsive element can be added to improve expression from the transgene by mobilizing RNA out of the nucleus and into the cytoplasm of cells;

cpPT—a Poly purine tract that facilitates second strand DNA synthesis prior to integration of the transgene into the host cell chromosome;

Promoter—a promoter initiates RNA transcription from the integrated transgene to express micro-RNA clusters (or other genetic elements of the construct), and in some embodiments, the vectors may use an EF-1 promoter;

Anti-CCR5—a micro RNA targeting messenger RNA for the host cell factor CCR5 to reduce its expression on the cell surface;

Anti-Rev/Tat—a micro RNA targeting HIV genomic or messenger RNA at the junction between HIV Rev and Tat coding regions, which is sometimes designated miRNA Tat or given a similar description in this application;

Anti-Vif—a micro RNA targeting HIV genomic or messenger RNA within the Vif coding region;

WPPE—a woodchuck hepatitis virus post-transcriptional regulatory element is an additional vector component that can be used to facilitate RNA transport of the nucleus; and

deltaU3 3'LTR—a modified version of a HIV 3' long terminal repeat where a portion of the U3 region has been deleted to improve safety of the vector.

One of ordinary skill in the art will recognize that the above components are merely examples, and that such components may be reorganized, substituted with other elements, or otherwise changed, so long as the construct is able to prevent expression of HIV genes and decrease the spread of infection.

Vectors of the invention may include either or both of the types of genetic cargo discussed above (i.e., genetic elements that direct expression of a gene or small RNAs, such as siRNA, shRNA, or miRNA that can prevent translation or transcription), and the vectors of the invention may also encode additionally useful products for the purpose of treatment or diagnosis of HIV. For instance, in some embodiments, these vectors may also encode green fluorescent protein (GFP) for the purpose of tracking the vectors or antibiotic resistance genes for the purposes of selectively maintaining genetically-modified cells in vivo.

The combination of genetic elements incorporated into the disclosed vectors is not particularly limited. For example, a vector herein may encode a single small RNA, two small RNAs, three small RNA, four small RNAs, five small RNAs, six small RNAs, seven small RNAs, eight small RNAs, nine small RNAs, or ten small RNAs, or eleven small RNAs, or twelve small RNAs. Such vectors may additionally encode other genetic elements to function in concert with the small RNAs to prevent expression and infection of HIV.

Those of ordinary skill in the art will understand that the therapeutic lentivirus may substitute alternate sequences for the promoter region, targeting of regulatory RNA, and types of regulatory RNA. Further, the therapeutic lentivirus of the disclosure may comprise changes in the plasmids used for packaging the lentivirus particles; these changes are required to increase levels of production in vitro.

Lentiviral Vector System

A lentiviral virion (particle) in accordance with various aspects and embodiments herein is expressed by a vector system encoding the necessary viral proteins to produce a virion (viral particle). In various embodiments, one vector containing a nucleic acid sequence encoding the lentiviral pol proteins is provided for reverse transcription and integration, operably linked to a promoter. In another embodiment, the pol proteins are expressed by multiple vectors. In other embodiments, vectors containing a nucleic acid sequence encoding the lentiviral Gag proteins for forming a

viral capsid, operably linked to a promoter, are provided. In embodiments, this gag nucleic acid sequence is on a separate vector than at least some of the pol nucleic acid sequence. In other embodiments, the gag nucleic acid is on a separate vector from all the pol nucleic acid sequences that encode pol proteins.

Numerous modifications can be made to the vectors herein, which are used to create the particles to further minimize the chance of obtaining wild type revertants. These include, but are not limited to deletions of the U3 region of the LTR, tat deletions and matrix (MA) deletions. In embodiments, the gag, pol and env vector(s) do not contain nucleotides from the lentiviral genome that package lentiviral RNA, referred to as the lentiviral packaging sequence.

The vector(s) forming the particle preferably do not contain a nucleic acid sequence from the lentiviral genome that expresses an envelope protein. Preferably, a separate vector that contains a nucleic acid sequence encoding an envelope protein operably linked to a promoter is used. This env vector also does not contain a lentiviral packaging sequence. In one embodiment the env nucleic acid sequence encodes a lentiviral envelope protein.

In another embodiment the envelope protein is not from the lentivirus, but from a different virus. The resultant particle is referred to as a pseudotyped particle. By appropriate selection of envelopes one can "infect" virtually any cell. For example, one can use an env gene that encodes an envelope protein that targets an endocytic compartment such as that of the influenza virus, VSV-G, alpha viruses (Semliki forest virus, Sindbis virus), arenaviruses (lymphocytic choriomeningitis virus), flaviviruses (tick-borne encephalitis virus, Dengue virus, hepatitis C virus, GB virus), rhabdoviruses (vesicular stomatitis virus, rabies virus), paramyxoviruses (mumps or measles) and orthomyxoviruses (influenza virus). Other envelopes that can preferably be used include those from Moloney Leukemia Virus such as MLV-E, MLV-A and GALV. These latter envelopes are particularly preferred where the host cell is a primary cell. Other envelope proteins can be selected depending upon the desired host cell. For example, targeting specific receptors such as a dopamine receptor can be used for brain delivery. Another target can be vascular endothelium. These cells can be targeted using a filovirus envelope. For example, the GP of Ebola, which by post-transcriptional modification become the GP₁ and GP₂ glycoproteins. In another embodiment, one can use different lentiviral capsids with a pseudotyped envelope (for example, FIV or SHIV [U.S. Pat. No. 5,654, 195]). A SHIV pseudotyped vector can readily be used in animal models such as monkeys.

Lentiviral vector systems as provided herein typically include at least one helper plasmid comprising at least one of a gag, pol, or rev gene. Each of the gag, pol and rev genes may be provided on individual plasmids, or one or more genes may be provided together on the same plasmid. In one embodiment, the gag, pol, and rev genes are provided on the same plasmid (e.g., FIGS. 4A-4B). In another embodiment, the gag and pol genes are provided on a first plasmid and the rev gene is provided on a second plasmid (e.g., FIGS. 5A-5B). Accordingly, both 3-vector and 4-vector systems can be used to produce a lentivirus as described herein. In embodiments, the therapeutic vector, at least one envelope plasmid and at least one helper plasmid are transfected into a packaging cell, for example a packaging cell line. A non-limiting example of a packaging cell line is the 293T/17 HEK cell line. When the therapeutic vector, the envelope

plasmid, and at least one helper plasmid are transfected into the packaging cell line, a lentiviral particle is ultimately produced.

In another aspect, a lentiviral vector system for expressing a lentiviral particle is disclosed. The system includes a lentiviral vector as described herein; an envelope plasmid for expressing an envelope protein optimized for infecting a cell; and at least one helper plasmid for expressing gag, pol, and rev genes, wherein when the lentiviral vector, the envelope plasmid, and the at least one helper plasmid are transfected into a packaging cell line, a lentiviral particle is produced by the packaging cell line, wherein the lentiviral particle is capable of inhibiting production of chemokine receptor CCR5 or targeting an HIV RNA sequence.

In another aspect, the lentiviral vector, which is also referred to herein as a therapeutic vector, includes the following elements: hybrid 5' long terminal repeat (RSV/5' LTR) (SEQ ID NOS: 34-35), Psi sequence (RNA packaging site) (SEQ ID NO: 36), RRE (Rev-response element) (SEQ ID NO: 37), cPPT (polypurine tract) (SEQ ID NO: 38), EF-1 α promoter (SEQ ID NO: 4), miR30CCR5 (SEQ ID NO: 1), miR21Vif (SEQ ID NO: 2), miR185Tat (SEQ ID NO: 3), Woodchuck Post-Transcriptional Regulatory Element (WPRE) (SEQ ID NOS: 32 or 80), and AU3 3' LTR (SEQ ID NO: 39). In another aspect, sequence variation, by way of substitution, deletion, addition, or mutation can be used to modify the sequences references herein.

In another aspect, a helper plasmid includes the following elements: CAG promoter (SEQ ID NO: 41); HIV component gag (SEQ ID NO: 43); HIV component pol (SEQ ID NO: 44); HIV Int (SEQ ID NO: 45); HIV RRE (SEQ ID NO: 46); and HIV Rev (SEQ ID NO: 47). In another aspect, the helper plasmid may be modified to include a first helper plasmid for expressing the gag and pol genes, and a second and separate plasmid for expressing the rev gene. In another aspect, sequence variation, by way of substitution, deletion, addition, or mutation can be used to modify the sequences references herein.

In another aspect, an envelope plasmid includes the following elements: RNA polymerase II promoter (CMV) (SEQ ID NO: 60) and vesicular stomatitis virus G glycoprotein (VSV-G) (SEQ ID NO: 62). In another aspect, sequence variation, by way of substitution, deletion, addition, or mutation can be used to modify the sequences references herein.

In various aspects, the plasmids used for lentiviral packaging are modified by substitution, addition, subtraction or mutation of various elements without loss of vector function. For example, and without limitation, the following elements can replace similar elements in the plasmids that comprise the packaging system: Elongation Factor-1 (EF-1), phosphoglycerate kinase (PGK), and ubiquitin C (UbC) promoters can replace the CMV or CAG promoter. SV40 poly A and bGH poly A can replace the rabbit beta globin poly A. The HIV sequences in the helper plasmid can be constructed from different HIV strains or clades. The VSV-G glycoprotein can be substituted with membrane glycoproteins from feline endogenous virus (RD114), gibbon ape leukemia virus (GALV), Rabies (FUG), lymphocytic choriomeningitis virus (LCMV), influenza A fowl plague virus (FPV), Ross River alphavirus (RRV), murine leukemia virus 10A1 (MLV), or Ebola virus (EboV).

Various lentiviral packaging systems can be acquired commercially (e.g., Lenti-vpak packaging kit from OriGene Technologies, Inc., Rockville, Md.), and can also be designed as described herein. Moreover, it is within the skill of a person ordinarily skilled in the art to substitute or

modify aspects of a lentiviral packaging system to improve any number of relevant factors, including the production efficiency of a lentiviral particle.

Bioassays

In various aspects, the present invention includes bioassays for determining the success of HIV treatment for achieving a functional cure. These assays provide a method for measuring the efficacy of the disclosed methods of immunization and treatment by measuring the frequency of transduced, HIV specific CD4 T cells in a patient. HIV-specific CD4 T cells are recognizable because, among others, they proliferate, change the composition of cell surface markers, induce signaling pathways including phosphorylation, and/or express specific marker proteins that may be cytokines, chemokines, caspases, phosphorylated signaling molecules or other cytoplasmic and/or nuclear components. Specific responding CD4 T cells are recognized for example, using labeled monoclonal antibodies or specific in situ amplification of mRNA sequences, that allow sorting of HIV-specific cells using flow cytometry sorting, magnetic bead separation or other recognized methods for antigen-specific CD4 T cell isolation. The isolated CD4 T cells are tested to determine the frequency of cells carrying integrated therapeutic lentivirus. Single cell testing methods may also be used including microfluidic separation of individual cells that are coupled with mass spectrometry, PCR, ELISA or antibody staining to confirm responsiveness to HIV and presence of integrated therapeutic lentivirus.

Thus, in various embodiments, following application of a treatment according to the invention (e.g., (a) immunization, (b) ex vivo leukocyte/lymphocyte culture; (c) re-stimulation with purified proteins, inactivated viruses, virally vectored proteins, bacterially vectored proteins, biological or chemical adjuvants including cytokines and/or chemokines, vehicles; and (d) infusion of the enriched, transduced T cells), a patient may be subsequently assayed to determine the efficacy of the treatment. A threshold value of target T cells in the body may be established to measure a functional cure at a determined value, for example, at about 1×10^8 HIV-specific CD4 T cells bearing genetic modification from therapeutic lentivirus. Alternatively, the threshold value may be about 1×10^5 , about 1×10^6 , about 1×10^7 , about 1×10^8 , about 1×10^9 , or about 1×10^{10} CD4 T cells in the body of the patient.

HIV-specific CD4 T cells bearing genetic modification from therapeutic lentivirus can be determined using any suitable method, such as but not limited to flow cytometry, cell sorting, FACS analysis, DNA cloning, PCR, RT-PCR or Q-PCR, ELISA, FISH, western blotting, southern blotting, high throughput sequencing, RNA sequencing, oligonucleotide primer extension, or other methods known in the art.

While methods for defining antigen specific T cells with genetic modifications are known in the art, utilizing such methods to combine identifying HIV-specific T cells with integrated or non-integrated gene therapy constructs as a standard measure for efficacy is a novel concept in the field of HIV treatment, as described variously herein.

Doses and Dosage Forms

The disclosed methods and compositions can be used for treating HIV+ patients during various stages of their disease. Accordingly, dosing regimens may vary based upon the condition of the patient and the method of administration.

In various embodiments, HIV-specific vaccines for the initial in vivo immunization are administered to a subject in need in varying doses. In general, vaccines delivered by intramuscular injection include about 10 μ g to about 300 μ g, about 25 μ g to about 275 μ g, about 50 μ g to about 250 μ g,

about 75 μ g to about 225, or about 100 μ g to about 200 μ g of HIV protein, either total virus protein prepared from inactivated virus particles, virus-like particles or purified virus protein from recombinant systems or purified from virus preparations. Recombinant viral or bacterial vectors may be administered by any and all of the routes described. Intramuscular vaccines will include about 1 μ g to about 100 μ g, about 10 μ g to about 90 μ g, about 20 μ g to about 80 μ g, about 30 μ g to about 70 μ g, about 40 μ g to about 60 μ g, or about 50 μ g of suitable adjuvant molecules and be suspended in oil, saline, buffer or water in volumes of 0.1 to 5 ml per injection dose, and may be soluble or emulsion preparations. Vaccines delivered orally, rectally, buccally, at genital mucosal or intranasally, including some virally-vectored or bacterially-vectored vaccines, fusion proteins, liposome formulations or similar preparations, may contain higher amounts of virus protein and adjuvant. Dermal, sub-dermal or subcutaneous vaccines utilize protein and adjuvant amounts more similar to oral, rectal or intranasal-delivered vaccines. Depending on responses to the initial immunization, vaccination may be repeated 1-5 times using the same or alternate routes for delivery. Intervals may be of 2-24 weeks between immunizations. Immune responses to vaccination are measured by testing HIV-specific antibodies in serum, plasma, vaginal secretions, rectal secretions, saliva or bronchoalveolar lavage fluids, using ELISA or similar methodology. Cellular immune responses are tested by in vitro stimulation with vaccine antigens followed by staining for intracellular cytokine accumulation followed by flow cytometry or similar methods including lymphoproliferation, expression of phosphorylated signaling proteins or changes in cell surface activation markers. Upper limits of dosing may be determined based on the individual patient and will depend on toxicity/safety profiles for each individual product or product lot.

Immunization may occur once, twice, three times, or repeatedly. For instance, an agent for HIV immunization may be administered to a subject in need once a week, once every other week, once every three weeks, once a month, every other month, every three months, every six months, every nine months, once a year, every eighteen months, every two years, every 36 months, or every three years.

Immunization will generally occur at least once before ex vivo expansion and enrichment of CD4 T cells, and immunization may occur once, twice, three times, or more after ex vivo leukocyte/lymphocyte culture/re-stimulation and infusion.

In one embodiment, HIV-vaccines for immunization are administered as a pharmaceutical composition. In one embodiment, the pharmaceutical composition comprising an HIV vaccine is formulated in a wide variety of nasal, pulmonary, oral, topical, or parenteral dosage forms for clinical application. Each of the dosage forms can comprise various disintegrating agents, surfactants, fillers, thickeners, binders, diluents such as wetting agents or other pharmaceutically acceptable excipients. The pharmaceutical composition comprising an HIV vaccine can also be formulated for injection.

HIV vaccine compositions for the purpose of immunization can be administered using any pharmaceutically acceptable method, such as intranasal, buccal, sublingual, oral, rectal, ocular, parenteral (intravenously, intradermally, intramuscularly, subcutaneously, intracisternally, intraperitoneally), pulmonary, intravaginal, locally administered, topically administered, topically administered after scarification, mucosally administered, via an aerosol, or via a buccal or nasal spray formulation.

Further, the HIV vaccine compositions can be formulated into any pharmaceutically acceptable dosage form, such as a solid dosage form, tablet, pill, lozenge, capsule, liquid dispersion, gel, aerosol, pulmonary aerosol, nasal aerosol, ointment, cream, semi-solid dosage form, and a suspension. Further, the composition may be a controlled release formulation, sustained release formulation, immediate release formulation, or any combination thereof. Further, the composition may be a transdermal delivery system.

In another embodiment, the pharmaceutical composition comprising an HIV vaccine is formulated in a solid dosage form for oral administration, and the solid dosage form can be powders, granules, capsules, tablets or pills. In yet another embodiment, the solid dosage form includes one or more excipients such as calcium carbonate, starch, sucrose, lactose, microcrystalline cellulose or gelatin. In addition, the solid dosage form can include, in addition to the excipients, a lubricant such as talc or magnesium stearate. In some embodiments, the oral dosage form is in immediate release or a modified release form. Modified release dosage forms include controlled or extended release, enteric release, and the like. The excipients used in the modified release dosage forms are commonly known to a person of ordinary skill in the art.

In a further embodiment, the pharmaceutical composition comprising a HIV vaccine is formulated as a sublingual or buccal dosage form. Such dosage forms comprise sublingual tablets or solution compositions that are administered under the tongue and buccal tablets that are placed between the cheek and gum.

In yet a further embodiment, the pharmaceutical composition comprising an HIV vaccine is formulated as a nasal dosage form. Such dosage forms of the present invention comprise solution, suspension, and gel compositions for nasal delivery.

In one embodiment, the pharmaceutical composition is formulated in a liquid dosage form for oral administration, such as suspensions, emulsions or syrups. In other embodiments, the liquid dosage form can include, in addition to commonly used simple diluents such as water and liquid paraffin, various excipients such as humectants, sweeteners, aromatics or preservatives. In particular embodiments, the composition comprising HIV vaccine or a pharmaceutically acceptable salt thereof is formulated to be suitable for administration to a pediatric patient.

In one embodiment, the pharmaceutical composition is formulated in a dosage form for parenteral administration, such as sterile aqueous solutions, suspensions, emulsions, non-aqueous solutions or suppositories. In other embodiments, the non-aqueous solutions or suspensions includes propyleneglycol, polyethyleneglycol, vegetable oils such as olive oil or injectable esters such as ethyl oleate. As a base for suppositories, witepsol, macrogol, tween 61, cacao oil, laurin oil or glycerinated gelatin can be used.

The dosage of the pharmaceutical composition can vary depending on the patient's weight, age, gender, administration time and mode, excretion rate, and the severity of disease.

For the purposes of re-stimulation, lymphocytes, PBMCs, and/or CD4 T cells are generally removed from a patient and isolated for re-stimulation and culturing. The isolated cells may be contacted with the same HIV vaccine or activating agent used for immunization or a different HIV vaccine or activating agent. In one embodiment, the isolated cells are contacted with about 10 ng to 5 μ g of an HIV vaccine or activating agent per about 10^6 cells in culture (or any other suitable amount). More specifically, the isolated cells may

be contacted with about 50 ng, about 100 ng, about 200 ng, about 300 ng, about 400 ng, about 500 ng, about 600 ng, about 700 ng, about 800 ng, about 900 ng, about 1 μ g, about 1.5 μ g, about 2 μ g, about 2.5 μ g, about 3 μ g, about 3.5 μ g, about 4 μ g, about 4.5 μ g, or about 5 μ g of an HIV vaccine or activating agent per about 10^6 cells in culture.

Activating agents or vaccines are generally used once for each in vitro cell culture but may be repeated after intervals of about 15 to about 35 days. For example, a repeat dosing could occur at about 15, about 16, about 17, about 18, about 19, about 20, about 21, about 22, about 23, about 24, about 25, about 26, about 27, about 28, about 29, about 30, about 31, about 32, about 33, about 34, or about 35 days.

For transduction of the enriched, re-stimulated cells, the cells may be transduced with lentiviral vectors or with other known vector systems as disclosed, for example, in FIGS. 4A-4C. The cells being transduced may be contacted with about 1-1,000 viral genomes (measured by RT-PCR assay of culture fluids containing lentivirus vector) per target cell in culture (or any other suitable amount). Lentivirus transduction may be repeated 1-5 times using the same range of 1-1,000 viral genomes per target cell in culture.

Cellular Enrichment

In various embodiments, cells such as T cells are obtained from an HIV infected patient and cultured. Culturing can occur in multiwell plates in a culture medium comprising conditioned media ("CM"). The levels of supernatant p24^{gag} ("p24") and viral RNA levels may be assessed by standard means. Those patients whose CM-cultured cells have peak p24 supernatant levels of less than 1 ng/ml may be suitable patients for large-scale T-cell expansion in CM with or without the use of additional anti-viral agents. Additionally, different drugs or drug combinations of interest may be added to different wells and the impact on virus levels in the sample may be assessed by standard means. Those drug combinations providing adequate viral suppression are therapeutically useful combinations. It is within the capacity of a competent technician to determine what constitutes adequate viral suppression in relation to a particular subject. In order to test the effectiveness of drugs of interest in limiting viral expansion, additional factors such as anti-CD3 antibodies may be added to the culture to stimulate viral production. Unlike culture methods for HIV infected cell samples known in the art, CM allows the culture of T cells for periods of over two months, thereby providing an effective system in which to assay long term drug effectiveness.

This approach allows the inhibition of gene expression driven by the HIV LTR promoter region in a cell population by the culture of cells in a medium comprising the CM. Culture in CM4 likely inhibits HIV LTR driven gene expression by altering one or more interactions between transcription mediating proteins and HIV gene expression regulatory elements. Transcription-mediating proteins of interest include host cell encoded proteins such as AP-1, NFkappaB, NF-AT, IRF, LEF-1 and Sp 1, and the HIV encoded protein Tat. HIV gene expression regulatory elements of interest include binding sites for AP-1, NFkappaB, NF-AT, IRF, LEF-1 and Sp1, as well as the transacting responsive element ("TAR") which interacts with Tat.

In a preferred embodiment, the HIV infected cells are obtained from a subject with susceptible transcription mediating protein sequences and susceptible HIV regulatory element sequences. In a more preferred embodiment, the HIV infected cells are obtained from a subject having wild-type transcription-mediating protein sequences and wild-type HIV regulatory sequences.

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Another method of enriching T Cells utilizes immunoaffinity-based selection. This method includes the simultaneous enrichment or selection of a first and second population of cells, such as a CD4+ and CD8+ cell population. Cells containing primary human T cells are contacted with a first immunoaffinity reagent that specifically binds to CD4 and a second immunoaffinity reagent that specifically binds to CD8 in an incubation composition, under conditions whereby the immunoaffinity reagents specifically bind to CD4 and CD8 molecules, respectively, on the surface of cells in the sample. Cells bound to the first and/or the second immunoaffinity reagent are recovered, thereby generating an enriched composition comprising CD4+ cells and CD8+ cells. This approach may include incubation of the composition with a concentration of the first and/or second immunoaffinity reagent that is at a sub-optimal yield concentration. Notably, in some embodiments, transduced cells are a mixed T cell population, and in other embodiments transduced cells are not a mixed T cell population.

In some embodiments, immunoaffinity-based selection is used where the solid support is a sphere, such as a bead, such as a microbead or nanobead. In other embodiments, the bead can be a magnetic bead. In another embodiment, the antibody contains one or more binding partners capable of forming a reversible bond with a binding reagent immobilized on the solid surface, such as a sphere or chromatography matrix, wherein the antibody is reversibly mobilized to the solid surface. In some embodiments, cells expressing a cell surface marker bound by the antibody on said solid surface are capable of being recovered from the matrix by disruption of the reversible binding between the binding reagent and binding partner. In some embodiments, the binding reagent is streptavidin or is a streptavidin analog or mutant.

Stable transduction of primary cells of the hematopoietic system and/or hematopoietic stem cells may be obtained by contacting, in vitro or ex vivo, the surface of the cells with both a lentiviral vector and at least one molecule which binds the cell surface. The cells may be cultured in a ventilated vessel comprising two or more layers under conditions conducive to growth and/or proliferation. In some embodiments, this approach may be used in conjunction with non-CD4+ T cell depletion and/or broad polyclonal expansion.

In another approach to T cell enrichment, PBMCs are stimulated with a peptide and enriched for cells secreting a cytokine, such as interferon-gamma. This approach generally involves stimulating a mixture of cells containing T cells with antigen, and effecting a separation of antigen-stimulated cells according to the degree to which they are labeled with the product. Antigen stimulation is achieved by exposing the cells to at least one antigen under conditions effective to elicit antigen-specific stimulation of at least one T cell. Labeling with the product is achieved by modifying the surface of the cells to contain at least one capture moiety, culturing the cells under conditions in which the product is secreted, released and specifically bound ("captured" or "entrapped") to said capture moiety; and labeling the captured product with a label moiety, where the labeled cells are not lysed as part of the labeling procedure or as part of the separation procedure. The capture moiety may incorporate detection of cell surface glycoproteins CD3 or CD4 to refine the enrichment step and increase the proportion of antigen-specific T cells in general, of CD4+ T cells in specific.

The following examples are given to illustrate aspects of the present invention. It should be understood, however, that the invention is not to be limited to the specific conditions

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or details described in these examples. All printed publications referenced herein are specifically incorporated by reference.

EXAMPLES

Example 1: Development of a Lentiviral Vector System

A lentiviral vector system was developed as summarized in FIG. 3 (linear form) and FIGS. 4A-C (circularized form). Referring first to the top portion of FIG. 3, a representative therapeutic vector has been designed and produced with the following elements being from left to right: hybrid 5' long terminal repeat (RSV/5' LTR) (SEQ ID NOS: 34-35), Psi sequence (RNA packaging site) (SEQ ID NO: 36), RRE (Rev-response element) (SEQ ID NO: 37), cPPT (polypurine tract) (SEQ ID NO: 38), EF-1 α promoter (SEQ ID NO: 4), miR30CCR5 (SEQ ID NO: 1), miR21Vif (SEQ ID NO: 2), miR185Tat (SEQ ID NO: 3), Woodchuck Post-Transcriptional Regulatory Element (WPPE) (SEQ ID NOS: 32 or 80), and AU3 3' LTR (SEQ ID NO: 39). The therapeutic vector detailed in FIG. 3 is also referred to herein as AGT103.

Referring next to the middle portion of FIG. 3, a helper plasmid has been designed and produced with the following elements being from left to right: CAG promoter (SEQ ID NO: 41); HIV component gag (SEQ ID NO: 43); HIV component pol (SEQ ID NO: 44); HIV Int (SEQ ID NO: 45); HIV RRE (SEQ ID NO: 46); and HIV Rev (SEQ ID NO: 47).

Referring next to the lower portion of FIG. 3, an envelope plasmid has been designed and produced with the following elements being from left to right: RNA polymerase II promoter (CMV) (SEQ ID NO: 60) and vesicular stomatitis virus G glycoprotein (VSV-G) (SEQ ID NO: 62).

Lentiviral particles were produced in 293T/17 HEK cells (purchased from American Type Culture Collection, Manassas, Va.) following transfection with the therapeutic vector, the envelope plasmid, and the helper plasmid (as shown in FIG. 3). The transfection of 293T/17 HEK cells, which produced functional viral particles, employed the reagent Poly(ethylenimine) (PEI) to increase the efficiency of plasmid DNA uptake. The plasmids and DNA were initially added separately in culture medium without serum in a ratio of 3:1 (mass ratio of PEI to DNA). After 2-3 days, cell medium was collected and lentiviral particles were purified by high-speed centrifugation and/or filtration followed by anion-exchange chromatography. The concentration of lentiviral particles can be expressed in terms of transducing units/ml (TU/ml). The determination of TU was accomplished by measuring HIV p24 levels in culture fluids (p24 protein is incorporated into lentiviral particles), measuring the number of viral DNA copies per cell by quantitative PCR, or by infecting cells and using light (if the vectors encode luciferase or fluorescent protein markers).

As mentioned above, a 3-vector system (i.e., a 2-vector lentiviral packaging system) was designed for the production of lentiviral particles. A schematic of the 3-vector system is shown in FIGS. 4A-4C. The schematic of FIGS. 4A-4C is a circularized version of the linear system previously described in FIG. 3. Briefly, and with reference to FIGS. 4A-4C, FIG. 4A depicts a helper plasmid, which, in this case, includes Rev. The vector appearing in FIG. 4B is the envelope plasmid. The vector appearing in FIG. 4C is the previously described therapeutic vector.

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Referring more specifically to FIG. 4A, the Helper plus Rev plasmid includes a CAG enhancer (SEQ ID NO: 40); a CAG promoter (SEQ ID NO: 41); a chicken beta actin intron (SEQ ID NO: 42); a HIV gag (SEQ ID NO: 43); a HIV Pol (SEQ ID NO: 44); a HIV Int (SEQ ID NO: 45); a HIV RRE (SEQ ID NO: 46); a HIV Rev (SEQ ID NO: 47); and a rabbit beta globin poly A (SEQ ID NO: 48).

The Envelope plasmid of FIG. 4B includes a CMV promoter (SEQ ID NO: 60); a beta globin intron (SEQ ID NO: 61); a VSV-G (SEQ ID NO: 62); and a rabbit beta globin poly A (SEQ ID NO: 63).

Synthesis of a 2-Vector Lentiviral Packaging System Including Helper (Plus Rev) and Envelope Plasmids.

Materials and Methods:

Construction of the Helper Plasmid:

The helper plasmid was constructed by initial PCR amplification of a DNA fragment from the pNL4-3 HIV plasmid (NIH Aids Reagent Program) containing Gag, Pol, and Integrase genes. Primers were designed to amplify the fragment with EcoRI and NotI restriction sites which could be used to insert at the same sites in the pCDNA3 plasmid (Invitrogen). The forward primer was (5'-TAAGCA-GAATTC ATGAATTGGCCAGGAAGAT-3') (SEQ ID NO: 81) and reverse primer was (5'-CCATACAATGAATGGA-CACTAGGCGGCCGCACGAAT-3') (SEQ ID NO: 82). The sequence for the Gag, Pol, Integrase fragment was as follows:

(SEQ ID NO: 83)

GAATTCATGAATTTGCCAGGAAGATGGAAACCAAAATGATAGGGGGAAAT
TGGAGGTTTTATCAAAGTAAGACAGTATGATCAGATACTCATAGAAATCT
GCGGACATAAAGCTATAGGTACAGTATTAGTAGGACCTACACCTGTCAAC
ATAATTGGAAGAAATCTGTTGACTCAGATTGGCTGCACCTTTAAATTTTCC
CATTAGTCCTATTGAGACTGTACCAGTAAATTAAGCCAGGAATGGATG
GCCCCAAAGTTAAACAATGGCCATTGACAGAAGAAAAATAAAGCATT
GTAGAAATTTGTACAGAAATGGAAAAGGAAGAAAAATTTCAAAATTTGG
GCCTGAAATCCATACAATACTCCAGTATTGGCCATAAAGAAAAAGACA
GTACTAAATGGAGAAAATTAGTAGATTTTCAGAGAACTTAATAAGAGAACT
CAAGATTTCTGGGAAGTTCAATTAGGAATACCACATCCTGCAGGGTTAAA
ACAGAAAAAATCAGTAACAGTACTGGATGTGGCGCATGATATTTTCAG
TTCCCTTAGATAAAGACTTCAGGAAGTATACTGCATTTACCATACCTAGT
ATAAACAATGAGACACCAGGGATTAGATATCAGTACAATGTGCTTCCACA
GGGATGGAAGGATCACCAGCAATATTCAGTGTAGCATGACAAAATCT
TAGAGCCTTTTAGAAAAAATAATCCAGACATAGTCATCTATCAATACATG
GATGATTTGTATGTAGGATCTGACTTAGAAATAGGGCAGCATAGAACAAA
AATAGAGGAAGTACAGACACATCTGTTGAGGTGGGATTTACCACACCAG
ACAAAAAATCAGAAAGAACCTCCATTCTTTGGATGGGTATGAATCTC
CATCTGATAAATGGACAGTACAGCCTATAGTGCTGCCAGAAAAGGACAG
CTGGACTGTCAATGACATACAGAAATTAGTGGGAAAATTGAATTGGGCAA
GTCAGATTTATGACAGGGATTAAAGTAAGGCAATTATGTAACCTCTTAGG
GGAACCAAGCACTAACAGAAAGTAGTACCACTAACAGAAAGCAGAGCT
AGAACTGGCAGAAAACAGGGAGATTCTAAAGAACCGGTACATGGAGTGT

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ATTATGACCCATCAAAAGACTTAATAGCAGAAATACAGAAGCAGGGGCAA
GGCCAATGGACATATCAAATTTATCAAGAGCCATTAAAAATCTGAAAAAC
AGGAAAGTATGCAAGAAATGAAGGGTGCCACACTAATGATGTGAACAAT
TAACAGAGGCAGTACAAAAAATAGCCACAGAAAGCATAGTAATATGGGGA
AAGACTCCTAAATTTAAATTACCCATACAAAAAGGAACATGGGAAGCATG
GTGGACAGAGTATTGGCAAGCCACCTGGATTCTGAGTGGGAGTTTGTCA
ATACCCCTCCCTTAGTGAAGTTATGGTACCAGTTAGAGAAAGAACCCATA
ATAGGAGCAGAACTTTCTATGTAGATGGGGCAGCCAATAGGGAACTAA
ATTAGGAAAAGCAGGATATGTAACAGACAGAGGAAGACAAAAGTTGTCC
CCCTAACGGACACAACAAATCAGAAGACTGAGTTACAAGCAATTCATCTA
GCTTTGCAGGATTCGGGATTAGAAGTAAACATAGTGACAGACTCACAATA
TGCATTGGGAATCATTCAAGCACAACCAGATAAGAGTGAATCAGAGTTAG
TCAGTCAAAATAATAGAGCAGTTAATAAAAAAGGAAAAAGTCTACCTGGCA
TGGGTACCAGCACACAAAGGAATTGGAGGAAATGAACAAGTAGATAAATT
GGTCAGTGTGGAATCAGGAAAGTACTATTTTTAGATGGAATAGATAAGG
CCCAAGAAGAACATGAGAAATATCACAGTAATTGGAGAGCAATGGCTAGT
GATTTTAACTTACCACCTGTAGTAGCAAAAGAAATAGTAGCCAGCTGTGA
TAAATGTCAGCTAAAAGGGGAGCCATGCATGGACAAGTAGACTGTAGCC
CAGGAATATGGCAGCTAGATTGTACACATTTAGAAGGAAAAGTTATCTTG
GTAGCAGTTTCATGTAGCCAGTGGATATATAGAAGCAGAAAGTAATCCAGC
AGAGACAGGGCAAGAAACAGCATACTTCTCTTAAATTAGCAGGAAGAT
GGCCAGTAAAAACAGTACATACAGACAATGGCAGCAATTTACCAGTACT
ACAGTTAAGGCCGCTGTGGTGGCGGGGATCAAGCAGGAATTTGGCAT
TCCCTACAATCCCCAAAGTCAAGGAGTAATAGAATCTATGAATAAAGAA
TAAAGAAAATTATAGGACAGGTAAGAGATCAGGCTGAACATCTTAAGACA
GCAGTACAAATGGCAGTATTTCATCCACAATTTTAAAGAAAAGGGGGAT
TGGGGGTACAGTGCAGGGGAAAGATAGTAGACATAATAGCAACAGACA
TACAAACTAAAGAAATTACAAAAACAAATTACAAAAATTCAAAATTTTCGG
GTTTATTACAGGGACAGCAGAGATCCAGTTTGGAAAGGACCAGCAAAGCT
CCTCTGGAAAGGTGAAGGGCAGTAGTAATACAAGATAATAGTGACATAA
AAGTAGTGCCAAGAGAAAGCAAGATCATCAGGGATTATGGAAAACAG
ATGGCAGGTGATGATTGTGTGGCAAGTAGACAGGATGAGGATTAA

Next, a DNA fragment containing the Rev, RRE, and rabbit beta globin poly A sequence with XbaI and XmaI flanking restriction sites was synthesized by MWG Operon. The DNA fragment was then inserted into the plasmid at the XbaI and XmaI restriction sites. The DNA sequence was as follows:

(SEQ ID NO: 84)

TCTAGAATGGCAGGAAGAAGCGGAGACAGCGACGAAGAGCTCATCAGAAC
AGTCAGACTCATCAAGCTTCTCTATCAAGCAACCCACCTCCCAATCCCCG
AGGGGACCCGACAGGCCCGAAGGAATAGAAGAAGAGGTGGAGAGAGAGA

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CAGAGACAGATCCATTGATTAGTGAACGGATCCTTGGCACTTATCTGGG
 ACGATCTGCGGAGCCTGTGCCTCTTCACTACACCGCTTGAGAGACTTA
 CTCTTGATTGTAAACGAGGATTGTGGAATCTTGGGACGACGGGGTGGGA
 AGCCCTCAAATATTGGTGAATCTCTACAATATTGGAGTCAGGAGCTAA
 AGAATAGAGGAGCTTTGTTCTTGGGTTCTTGGGAGCAGCAGGAAGCACT
 ATGGGCGCAGCGTCAATGACGCTGACGCTACAGGCCAGACAATTATTGTC
 TGGTATAGTGACGACGAGAACAATTGCTGAGGGCTATTGAGGCGCAAC
 AGCATCTGTTGCAACTCACAGTCTGGGGCATCAAGCAGCTCCAGGCAAGA
 ATCTTGGCTGTGGAAGATACCTAAAGGATCAACAGCTCCTAGATCTTTT
 TCCCTCTGCCAAAAATTATGGGACATCATGAAGCCCTTGAGCATCTGA
 CTTCTGGCTAATAAGGAAATTTATTTTCAATTGCAATAGTGTGTGGAAT
 TTTTGTGTCTCTCACTCGAAGGACATATGGGAGGGCAATCATTTAAA
 ACATCAGAATGAGTATTTGGTTAGAGTTTGGCAACATATGCCATATGCT
 GGCTGCCATGAACAAAGGTGGCTATAAAGAGGTATCAGTATATGAAACA
 GCCCCCTGCTGTCATTCTTATTTCCATAGAAAAGCCTTGACTTGAGGTT
 AGATTTTTTTTATATTTTGTGTTTGTGTTATTTTTCTTTAACATCCCTA
 AAATTTTCCTTACATGTTTTACTAGCCAGATTTTTCTCTCTCTCTGACT
 ACTCCAGTCATAGCTGTCCCTCTTCTCTTATGAAGATCCCTCGACCTGC
 AGCCCAAGCTTGGCGTAATCATGGTCATAGCTGTTTCTGTGTGAAATTG
 TTATCCGCTCACAATTCCACACAACATACGAGCCGGAAGCATAAAGTGTA
 AAGCCTGGGGTGCCTAATGAGTGAGCTAACTCACATTAATTGCGTTGCGC
 TCACTGCCCGCTTTCCAGTCGGGAAACCTGTCGTGCCAGCGGATCCGCAT
 CTCAATTAGTCAGCAACCATAGTCCCGCCCCCTAACTCCGCCCATCCCGCC
 CCTAACTCCGCCCAGTTCGCCCCATTCTCCGCCCATGGCTGACTAATTT
 TTTTATTTATGACAGAGCCGAGGCCGCTCGGCCTCTGAGCTATTCCAG
 AAGTAGTGAGGAGGCTTTTTTGGAGGCCTAGGCTTTTGCAAAAAGCTAAC
 TTGTTTATTGACGCTTATAATGGTTACAAATAAGCAATAGCATCACAAA
 TTTCACAAATAAGCATTTTTTCTACTGCATTCTAGTTGTGTTTGTCCA
 AACTCATCAATGTATCTTATCAGCGGCCGCCCGGG

Finally, the CMV promoter of pCDNA3.1 was replaced
 with the CAG enhancer/promoter plus a chicken beta actin
 intron sequence. A DNA fragment containing the CAG
 enhancer/promoter/intron sequence with MluI and EcoRI
 flanking restriction sites was synthesized by MWG Operon.
 The DNA fragment was then inserted into the plasmid at the
 MluI and EcoRI restriction sites. The DNA sequence was as
 follows:

(SEQ ID NO: 85)

ACGCGTTAGTTATTAATAGTAATCAATTACGGGGTCATTAGTTATAGCC
 CATATATGGAGTTCGCGTTACATAACTTACGGTAAATGGCCCGCTGGC
 TGACCGCCCAACGACCCCGCCCATTTGACGTCAATAATGACGTATGTTCC
 CATAGTAACGCCAATAGGGACTTTCCATTGACGTCAATGGGTGGACTATT
 TACGGTAAACTGCCCACTTGGCAGTACATCAAGTGTATCATATGCCAAGT

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ACGCCCCCTATTGACGTCAATGACGGTAAATGGCCCCCTTGGCATTATGC
 CCAGTACATGACCTTATGGGACTTTCTTACTTGGCAGTACATCTACGTAT
 TAGTCATCGCTATTACCATTGGGTGAGGTGAGCCCCACGTTCTGCTTAC
 TCTCCCCATCTCCCCCCCCCTCCCCACCCCCAATTTGTATTATTATTATT
 TTTAATTATTTTGTGTCAGCGATGGGGCGGGGGGGGGGGGGCGCGCGC
 AGGCGGGGCGGGGCGGGGCGAGGGGCGGGGCGGGGCGAGGCGGAGAGGTG
 CGGCGGCAGCCAATCAGAGCGGCGCTCCGAAAGTTTCTTTTATGGCG
 AGGCGGGCGGGGCGGGCGGCTATAAAAAGCGAAGCGCGCGGGCGGGG
 AGTCGCTGCGTTGCCCTTCGCCCGGTGCCCGCTCCGCGCGGCTCGCGCC
 GCGCGCGCGGCTCTGACTGACCGGTTACTCCACAGGTGAGCGGGCGG
 GACGGCCCTTCTCTCCGGGCTGTAATTAGCGCTTGGTTTAAAGACGGCT
 CGTTTCTTTTCTGTGGCTGCGTGAAAGCCTTAAAGGGCTCCGGGAGGGCC
 CTTTGTGCGGGGGGAGCGGCTCGGGGGGTGCGTGCCTGTGTGTGTGCGT
 GGGGAGCGCCGCGTGCAGCCCGCGCTGCCCGGCGGCTGTGAGCGCTGCGG
 GCGCGCGCGGGGCTTTGTGCGCTCCGCGTGTGCGCGAGGGGAGCGCGGC
 CGGGGGCGGTGCCCGCGGTGCGGGGGGTGCGAGGGGGAACAAAGGCTG
 CGTGCGGGGTGTGTGCGTGGGGGGGTGAGCAGGGGGTGTGGGCGCGCGG
 TCGGGCTGTAACCCCCCTGCACCCCCCTCCCCGAGTTGCTGAGCACGG
 CCGGCTTCGGGTGCGGGGCTCCGTGCGGGGCGTGGCGCGGGGCTCGCCG
 TGCCGGGCGGGGGGTGGCGGCAGGTGGGGGTGCCGGGCGGGGCGGGGCCG
 CCTCGGGCGGGGAGGGCTCGGGGAGGGGCGCGGCGGCCCCGAGCGCC
 GGCGGCTGTGAGGCGCGGCGAGCGCAGCCATTGCCTTTTATGGTAATC
 GTGCGAGAGGGCGCAGGGACTTCTTTGTCCCAATCTGGCGGAGCCGAA
 ATCTGGGAGGCGCGCCCGCACCCCCCTCTAGCGGGCGCGGCGAAGCGGTG
 CGGCGCGCGGAGGAAGGAAATGGGCGGGGAGGGCTTCGTGCGTGCCTGC
 GCGCGCTCCCTTCTCCATCTCCAGCCTCGGGGTGCCGAGGGGGACG
 GCTGCCTTCGGGGGGGACGGGCGAGGGCGGGTTCGGCTTCTGCGTGTG
 ACCGCGCGGAATTC

Construction of the VSV-G Envelope Plasmid:

The vesicular stomatitis Indiana virus glycoprotein (VSV-
 G) sequence was synthesized by MWG Operon with flank-
 ing EcoRI restriction sites. The DNA fragment was then
 inserted into the pCDNA3.1 plasmid (Invitrogen) at the
 EcoRI restriction site and the correct orientation was deter-
 mined by sequencing using a CMV specific primer. The
 DNA sequence was as follows:

(SEQ ID NO: 86)

GAATTCATGAAGTGCCTTTTGTACTTAGCCTTTTATTGCGGTGAA
 TTGCAAGTTCACCATAGTTTTTCCACACAACCAAAAGGAACTGGAAAA
 ATGTTCTTCTAATTACCATTATTGCCGCTCAAGCTCAGATTTAAATTGG
 CATAATGACTTAATAGGCACAGCCTTACAAGTCAAAATGCCCAAGAGTCA
 CAAGGCTATTCAAGCAGACGGTTGGATGTGTATGCTTCCAAATGGGTCA
 CTACTTGTGATTTCCGCTGGTATGGACCGAAGTATATAACACATTCCATC

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CGATCCTTCACTCCATCTGTAGAACAATGCAAGGAAAGCATTGAACAAAC
 GAAACAAGGAACCTGGCTGAATCCAGGCTTCCCTCCTCAAAGTTGTGGAT
 ATGCAACTGTGACGGATGCCGAAGCAGTGATTGTCCAGGTGACTCCTCAC
 CATGTGCTGGTTGATGAATACACAGGAGAATGGGTTGATTCACAGTTCAT
 CAACGGAAAAATGCAGCAATTACATATGCCCCACTGTCCATAACTCTACAA
 CCTGGCATTCTGACTATAAGGTCAAAGGGCTATGTGATTCTAACCTCATT
 TCCATGGACATCACCTTCTTCTCAGAGGACGGAGAGCTATCATCCCTGGG
 AAAGGAGGGCACAGGGTTCAGAAGTAACTACTTTGCTTATGAACTGGAG
 GCAAGGCCTGCAAAATGCAATACTGCAAGCATTGGGGAGTCAGACTCCCA
 TCAGGTGTCTGGTTCGAGATGGCTGATAAGGATCTCTTTGTGCGCCAG
 ATTCCCTGAATGCCCAGAAGGGTCAAGTATCTCTGCTCCATCTCAGACCT
 CAGTGGATGTAAGTCTAATTCAGGACGTTGAGAGGATCTTGGATTATTC
 CTCTGCCAAGAAACCTGGAGCAAAATCAGAGCGGGTCTTCCAATCTCTCC
 AGTGGATCTCAGCTATCTTGCTCTAAAAACCCAGGAACCGGTCTTGCTT
 TCACCATAATCAATGGTACCCTAAATACTTTGAGACCAGATACATCAGA
 GTCGATATTGTCTGCCAATCTCTCAAGAATGGTCGAATGATCAGTGG
 AACTACCACAGAAAGGGAACCTGTGGGATGACTGGGCACCATATGAAGACG
 TGGAAATTGGACCCAATGGAGTTCTGAGGACCAGTTCAGGATATAAGTTT
 CCTTTATACATGATTGGACATGGTATGTTGGACTCCGATCTTCATCTTAG
 CTCAAAGGCTCAGGTGTTTCAACATCCTCACATTCAAGACGCTGCTTCGC
 AACTTCTGATGATGAGAGTTTATTTTTTGGTGATACTGGGCTATCCAAA
 AATCCAATCGAGCTTGTAGAAGTTGGTTCAGTAGTTGGAAGTCTCTAT
 TGCTCTTTTTTTCTTTATCATAGGGTTAATCATTGGACTATTCTTGGTTC
 TCCGAGTTGGTATCCATCTTTGCATTAAATTAAAGCACACCAAGAAAAAGA
 CAGATTTATACAGACATAGAGATGAGAATTC

A 4-vector system (i.e., a 3-vector lentiviral packaging system) has also been designed and produced using the methods and materials described herein. A schematic of the 4-vector system is shown in FIGS. 5A-5D. Briefly, and with reference to FIG. 5, the vector of FIG. 5A is a helper plasmid, which, in this case, does not include Rev. The vector depicted in FIG. 5B is a separate Rev plasmid. The vector depicted in FIG. 5C is the envelope plasmid. The vector depicted in FIG. 5D is the previously described therapeutic vector.

Referring, in part, to FIG. 5A, the Helper plasmid includes a CAG enhancer (SEQ ID NO: 49); a CAG promoter (SEQ ID NO: 50); a chicken beta actin intron (SEQ ID NO: 51); a HIV gag (SEQ ID NO: 52); a HIV Pol (SEQ ID NO: 53); a HIV Int (SEQ ID NO: 54); a HIV RRE (SEQ ID NO: 55); and a rabbit beta globin poly A (SEQ ID NO: 56).

The Rev plasmid depicted in FIG. 5B includes a RSV promoter (SEQ ID NO: 57); a HIV Rev (SEQ ID NO: 58); and a rabbit beta globin poly A (SEQ ID NO: 59).

The Envelope plasmid depicted in FIG. 5C includes a CMV promoter (SEQ ID NO: 60); a beta globin intron (SEQ ID NO: 61); a VSV-G (SEQ ID NO: 62); and a rabbit beta globin poly A (SEQ ID NO: 63).

Synthesis of a 3-Vector Lentiviral Packaging System Including Helper, Rev, and Envelope Plasmids.

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Materials and Methods:

Construction of the Helper Plasmid without Rev:

The Helper plasmid without Rev was constructed by inserting a DNA fragment containing the RRE and rabbit beta globin poly A sequence. This sequence was synthesized by MWG Operon with flanking XbaI and XmaI restriction sites. The RRE/rabbit poly A beta globin sequence was then inserted into the Helper plasmid at the XbaI and XmaI restriction sites. The DNA sequence is as follows:

(SEQ ID NO: 87)
 TCTAGAAGGAGCTTTGTTCTTGGGTTCTTGGGAGCAGCAGGAAGCACTA
 TGGGCGCAGCGTCAATGACGCTGACGGTACAGGCCAGACAATTATTGTCT
 GGTATAGTGCAGCAGCAGAACAATTTGCTGAGGGCTATTGAGGCGCAACA
 GCATCTGTTGCAACTCACAGTCTGGGGCATCAAGCAGCTCCAGGCAAGAA
 TCCTGGCTGTGGAAGATACCTAAAGGATCAACAGCTCCTAGATCTTTTT
 CCCTCTGCCAAAAATTTATGGGGACATCATGAAGCCCTTGAGCATCTGAC
 TTCTGGCTAATAAAGGAAATTTATTTTCATTGCAATAGTGTGTGGAATT
 TTTTGTGTCTCTCACTCGGAAGGACATATGGGAGGGCAATCATTTAAAA
 CATCAGAATGAGTATTTGGTTTAGAGTTTGGCAACATATGCCATATGCTG
 GCTGCCATGAACAAAGGTGGCTATAAAGAGGTCATCAGTATATGAACAG
 CCCCCTGCTGTCCATTCTTATTCATAGAAAAGCCTTGACTTGAGGTTA
 GATTTTTTTTATATTTTGTGTTATTTTTTCTTTAACATCCCTAA
 AATTTTCTTACATGTTTACTAGCCAGATTTTCTCCTCTCCTGACTA
 CTCCAGTCATAGCTGTCCCTCTCTCTATGAAGATCCCTCGACCTGCA
 GCCCAAGCTTGGCGTAATCATGGTCATAGCTGTTTCTGTGTGAAATGT
 TATCCGCTCACAATTCACACAACATACGAGCCGAAGCATAAAGTGTA
 AGCCTGGGGTGCCTAATGAGTGAGCTAACTCACATTAATTGCGTTGCGCT
 CACTGCCCGCTTTCCAGTCGGGAAACCTGTCGTGCCAGCGGATCCGCATC
 TCAATTAGTCAGCAACCATAGTCCCGCCCTAACTCCGCCCATCCGCCCC
 CTAATCCGCCAGTTCGCCCTTCTCCGCCCATGGCTGACTAATTTT
 TTTTATTTATGCAGAGGCCGAGGCCCTCGGCCCTCTGAGCTATTCCAGA
 AGTAGTGAGGAGGCTTTTGGAGGCTAGGCTTTTGCAAAAGCTAACT
 TGTTTTATGCGCTTATAATGGTTACAAATAAAGCAATAGCATCACAAT
 TTCACAAATAAAGCATTTTTTTCTACTGCATTCTAGTTGTGGTTTGCCAA
 ACTCATCAATGTATCTTATCACCCTGGG

Construction of the Rev Plasmid:

The RSV promoter and HIV Rev sequence was synthesized as a single DNA fragment by MWG Operon with flanking MfeI and XbaI restriction sites. The DNA fragment was then inserted into the pCDNA3.1 plasmid (Invitrogen) at the MfeI and XbaI restriction sites in which the CMV promoter is replaced with the RSV promoter. The DNA sequence was as follows:

(SEQ ID NO: 88)
 CAATTGCGATGTACGGGCCAGATATACGCGTATCTGAGGGGACTAGGGTG
 TGTTTAGGCGAAAAGCGGGGCTTCGGTTGTACGCGGTTAGGAGTCCCCTC

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AGGATATAGTAGTTTCGCTTTTGCATAGGGAGGGGAAATGTAGTCTTAT
 GCAATACACTTGTAGTCTTGCACATGGTAACGATGAGTTAGCAACATGC
 CTTACAAGGAGAGAAAAAGCACCGTGCGATGCGGATTGGTGAAGTAAGGT
 GGTACGATCGTGCCCTTATTAGGAAGGCAACAGACAGGTCTGACATGGATT
 GGACGAACCACTGAATTCGCGATTGCGAGAGATAATTGTATTTAAGTGCCT
 AGCTCGATACAATAACGCCATTTGACCATTACACACATTGGTGTGCACC
 TCCAAGCTCGAGCTCGTTTAGTGAACCGTCAGATCGCCTGGAGACGCCAT
 CCACGCTGTTTTGACCTCCATAGAAGACACCGGACCGATCCAGCCTCCC
 CTCGAAGCTAGCGATTAGGCATCTCCTATGGCAGGAAGAAGCGGAGACAG
 CGACGAAGAACTCCTCAAGGCAGTCAGACTCATCAAGTTTCTCTATCAA
 GCAACCCACCTCCCAATCCCGAGGGGACCCGACAGGCCGGAAGGAATAGA
 AGAAGAAGGTGGAGAGAGAGACAGAGACAGATCCATTGATTAGTGAACG
 GATCCTTAGCACTTATCTGGGACGATCTGCGGAGCCTGTGCTCTTCAGC
 TACCACCGCTTGAGAGACTTACTCTTGATTGTAACGAGGATTGTGGAAC
 TCTGGGACGCGAGGGGTGGGAAGCCCTCAAATATTGGTGAATCTCCTAC
 AATATTGGAGTCAGGAGCTAAGAATAGTCTAGA

The plasmids for the 2-vector and 3-vector packaging systems could be modified with similar elements and the intron sequences could potentially be removed without loss of vector function. For example, the following elements could replace similar elements in the 2-vector and 3-vector packaging system:

Promoters: Elongation Factor-1 (EF-1) (SEQ ID NO: 64), phosphoglycerate kinase (PGK) (SEQ ID NO: 65), and ubiquitin C (UbC) (SEQ ID NO: 66) can replace the CMV (SEQ ID NO: 60) or CAG promoter (SEQ ID NO: 100). These sequences can also be further varied by addition, substitution, deletion or mutation.

Poly A sequences: SV40 poly A (SEQ ID NO: 67) and bGH poly A (SEQ ID NO: 68) can replace the rabbit beta globin poly A (SEQ ID NO: 48). These sequences can also be further varied by addition, substitution, deletion or mutation.

HIV Gag, Pol, and Integrase sequences: The HIV sequences in the Helper plasmid can be constructed from different HIV strains or clades. For example, HIV Gag (SEQ ID NO: 69); HIV Pol (SEQ ID NO: 70); and HIV Int (SEQ ID NO: 71) from the Bal strain can be interchanged with the gag, pol, and int sequences contained in the helper/helper plus Rev plasmids as outlined herein. These sequences can also be further varied by addition, substitution, deletion or mutation.

Envelope: The VSV-G glycoprotein can be substituted with membrane glycoproteins from feline endogenous virus (RD114) (SEQ ID NO: 72), gibbon ape leukemia virus (GALV) (SEQ ID NO: 73), Rabies (FUG) (SEQ ID NO: 74), lymphocytic choriomeningitis virus (LCMV) (SEQ ID NO: 75), influenza A fowl plague virus (FPV) (SEQ ID NO: 76), Ross River alphavirus (RRV) (SEQ ID NO: 77), murine leukemia virus 10A1 (MLV) (SEQ ID NO: 78), or Ebola virus (EboV) (SEQ ID NO: 79). Sequences for these envelopes are identified in the sequence portion herein. Further, these sequences can also be further varied by addition, substitution, deletion or mutation.

In summary, the 3-vector versus 4-vector systems can be compared and contrasted, in part, as follows. The 3-vector

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lentiviral vector system contains: 1. Helper plasmid: HIV Gag, Pol, Integrase, and Rev/Tat; 2. Envelope plasmid: VSV-G/FUG envelope; and 3. Therapeutic vector: RSV 5'LTR, Psi Packaging Signal, Gag fragment, RRE, Env fragment, cPPT, WPRE, and 3'delta LTR. The 4-vector lentiviral vector system contains: 1. Helper plasmid: HIV Gag, Pol, and Integrase; 2. Rev plasmid: Rev; 3. Envelope plasmid: VSV-G/FUG envelope; and 4. Therapeutic vector: RSV 5'LTR, Psi Packaging Signal, Gag fragment, RRE, Env fragment, cPPT, WPRE, and 3'delta LTR. Sequences corresponding with the above elements are identified in the sequence listings portion herein.

Example 2: Development of an Anti-HIV Lentivirus Vector

The purpose of this example was to develop an anti-HIV lentivirus vector.

Inhibitory RNA Designs.

The sequence of *Homo sapiens* chemokine C—C motif receptor 5 (CCR5) (GC03P046377) mRNA was used to search for potential siRNA or shRNA candidates to knock-down CCR5 levels in human cells. Potential RNA interference sequences were chosen from candidates selected by siRNA or shRNA design programs such as from the Broad Institute or the BLOCK-iT RNAi Designer from Thermo Scientific. Individual selected shRNA sequences were inserted into lentiviral vectors immediately 3' to a RNA polymerase III promoter such as H1, U6, or 7SK to regulate shRNA expression. These lentivirus-shRNA constructs were used to transduce cells and measure the change in specific mRNA levels. The shRNA most potent for reducing mRNA levels were embedded individually within a microRNA backbone to allow for expression by either the CMV or EF-1alpha RNA polymerase II promoters. The microRNA backbone was selected from mirbase.org. RNA sequences were also synthesized as synthetic siRNA oligonucleotides and introduced directly into cells without using a lentiviral vector.

The genomic sequence of Bal strain of human immunodeficiency virus type 1 (HIV-1 85US_BaL, accession number AY713409) was used to search for potential siRNA or shRNA candidates to knockdown HIV replication levels in human cells. Based on sequence homology and experience, the search focused on regions of the Tat and Vif genes of HIV although an individual of skill in the art will understand that use of these regions is non-limiting and other potential targets might be selected. Importantly, highly conserved regions of gag or pol genes could not be targeted by shRNA because these same sequences were present in the packaging system complementation plasmids needed for vector manufacturing. As with the CCR5 (NM 000579.3, NM 001100168.1-specific) RNAs, potential HIV-specific RNA interference sequences were chosen from candidates selected by siRNA or shRNA design programs such as from the Gene-E Software Suite hosted by the Broad Institute (broadinstitute.org/mai/public) or the BLOCK-iT RNAi Designer from Thermo Scientific (madesigner.thermofisher.com/rnaexpress/

setOption.do?designOption=shrna&pid=6712627360706061801). Individual selected shRNA sequences were inserted into lentiviral vectors immediately 3' to a RNA polymerase III promoter such as H1, U6, or 7SK to regulate shRNA expression. These lentivirus-shRNA constructs were used to transduce cells and measure the change in specific mRNA levels. The shRNA most potent for reducing mRNA levels were embedded individually within a microRNA

backbone to allow for expression by either the CMV or EF-1alpha RNA polymerase II promoters

Vector Constructions.

For CCR5, Tat or Vif shRNA, oligonucleotide sequences containing BamHI and EcoRI restriction sites were synthesized by Eurofins MWG Operon, LLC. Overlapping sense and antisense oligonucleotide sequences were mixed and annealed during cooling from 70 degrees Celsius to room temperature. The lentiviral vector was digested with the restriction enzymes BamHI and EcoRI for one hour at 37 degrees Celsius. The digested lentiviral vector was purified by agarose gel electrophoresis and extracted from the gel using a DNA gel extraction kit from Invitrogen. The DNA concentrations were determined and vector to oligo (3:1 ratio) were mixed, allowed to anneal, and ligated. The ligation reaction was performed with T4 DNA ligase for 30 minutes at room temperature. 2.5 microliters of the ligation mix were added to 25 microliters of STBL3 competent bacterial cells. Transformation was achieved after heat-shock at 42 degrees Celsius. Bacterial cells were spread on agar plates containing ampicillin and drug-resistant colonies (indicating the presence of ampicillin-resistance plasmids) were recovered, purified and expanded in LB broth. To check for insertion of the oligo sequences, plasmid DNA were extracted from harvested bacteria cultures with the Invitrogen DNA mini prep kit. Insertion of the shRNA sequence in the lentiviral vector was verified by DNA sequencing using a specific primer for the promoter used to regulate shRNA expression. Exemplary vector sequences that were determined to restrict HIV replication can be found in FIG. 6. For example, the shRNA sequences with the highest activity against CCR5, Tat or Vif gene expression were then assembled into a microRNA (miR) cluster under control of the EF-1alpha promoter. The promoter and miR sequences are depicted in FIG. 6.

Further, and using standard molecular biology techniques (e.g., Sambrook; Molecular Cloning: A Laboratory Manual, 4th Ed.) as well as the techniques described herein, a series of lentiviral vectors have been developed as depicted in FIG. 7 herein.

Vector 1 was developed and contains, from left to right: a long terminal repeat (LTR) portion (SEQ ID NO: 35); a H1 element (SEQ ID NO: 101); a shCCR5 (SEQ ID NOS: 16, 18, 20, 22, or 24-Y); a posttranscriptional regulatory element of woodchuck hepatitis virus (WPRE) (SEQ ID NOS: 32, 80); and a long terminal repeat portion (SEQ ID NO: 102).

Vector 2 was developed and contains, from left to right: a long terminal repeat (LTR) portion (SEQ ID NO: 35); a H1 element (SEQ ID NO: 101); a shRev/Tat (SEQ ID NO: 10); a H1 element (SEQ ID NO: 101); a shCCR5 (SEQ ID NOS: 16, 18, 20, 22, or 24); a posttranscriptional regulatory element of woodchuck hepatitis virus (WPRE) (SEQ ID NOS: 32, 80); and a long terminal repeat portion (SEQ ID NO: 102).

Vector 3 was developed and contains, from left to right: a long terminal repeat (LTR) portion (SEQ ID NO: 35); a H1 element (SEQ ID NO: 101); a shGag (SEQ ID NO: 12); a H1 element (SEQ ID NO: 101); a shCCR5 (SEQ ID NOS: 16, 18, 20, 22, or 24); a posttranscriptional regulatory element of woodchuck hepatitis virus (WPRE) (SEQ ID NOS: 32, 80); and a long terminal repeat portion (SEQ ID NO: 102).

Vector 4 was developed and contains, from left to right: a long terminal repeat (LTR) portion (SEQ ID NO: 35); a 7SK element (SEQ ID NO: 103); a shRev/Tat (SEQ ID NO: 10); a H1 element (SEQ ID NO: 101); a shCCR5 (SEQ ID NOS: 16, 18, 20, 22, or 24); a posttranscriptional regulatory

element of woodchuck hepatitis virus (WPRE) (SEQ ID NOS: 32, 80); and a long terminal repeat portion (SEQ ID NO: 102).

Vector 5 was developed and contains, from left to right: a long terminal repeat (LTR) portion (SEQ ID NO: 35); a EF1 element (SEQ ID NO: 4); miR30CCR5 (SEQ ID NO: 1); MiR21Vif (SEQ ID NO: 2); miR185Tat (SEQ ID NO: 3); a posttranscriptional regulatory element of woodchuck hepatitis virus (WPRE) (SEQ ID NOS: 32, 80); and a long terminal repeat portion (SEQ ID NO: 102).

Vector 6 was developed and contains, from left to right: a long terminal repeat (LTR) portion (SEQ ID NO: 35); a EF1 element (SEQ ID NO: 4); miR30CCR5 (SEQ ID NO: 1); MiR21Vif (SEQ ID NO: 2); miR155Tat (SEQ ID NO: 104); a posttranscriptional regulatory element of woodchuck hepatitis virus (WPRE) (SEQ ID NOS: 32, 80); and a long terminal repeat portion (SEQ ID NO: 102).

Vector 7 was developed and contains, from left to right: a long terminal repeat (LTR) portion (SEQ ID NO: 35); a EF1 element (SEQ ID NO: 4); miR30CCR5 (SEQ ID NO: 1); MiR21Vif (SEQ ID NO: 2); miR185Tat (SEQ ID NO: 3); a posttranscriptional regulatory element of woodchuck hepatitis virus (WPRE) (SEQ ID NOS: 32, 80); and a long terminal repeat portion (SEQ ID NO: 102).

Vector 8 was developed and contains, from left to right: a long terminal repeat (LTR) portion (SEQ ID NO: 35); a EF1 element (SEQ ID NO: 4); miR30CCR5 (SEQ ID NO: 1); MiR21Vif (SEQ ID NO: 2); miR185Tat (SEQ ID NO: 3); and a long terminal repeat portion (SEQ ID NO: 102).

Vector 9 was developed and contains, from left to right: a long terminal repeat (LTR) portion (SEQ ID NO: 35); a CD4 element (SEQ ID NO: 30); miR30CCR5 (SEQ ID NO: 1); miR21Vif (SEQ ID NO: 2); miR185Tat (SEQ ID NO: 3); a posttranscriptional regulatory element of woodchuck hepatitis virus (WPRE) (SEQ ID NOS: 32, 80); and a long terminal repeat portion (SEQ ID NO: 102).

Development of Vectors

It should be noted that not all vectors developed for these experiments necessarily worked as might be predicted. More specifically, a lentivirus vector against HIV might include three main components: 1) inhibitory RNA to reduce the level of HIV binding proteins (receptors) on the target cell surface to block initial virus attachment and penetration; 2) overexpression of the HIV TAR sequence that will sequester viral Tat protein and decrease its ability to transactivate viral gene expression; and 3) inhibitory RNA that attack important and conserved sequences within the HIV genome.

With respect to the first point above, a key cell surface HIV binding protein is the chemokine receptor CCR5. HIV particles attach to susceptible T cells by binding to the CD4 and CCR5 cell surface proteins. Because CD4 is an essential glycoprotein on the cell surface that is important for the immunological function of T cells, this was not chosen as a target to manipulate its expression levels. However, people born homozygous for null mutations in the CCR5 gene and completely lacking receptor expression, live normal lives save for enhanced susceptibility to a few infectious diseases and the possibility of developing rare autoimmunity. Thus, modulating CCR5 was determined to be a relatively safe approach and was a primary target in the development of anti-HIV lentivirus vectors.

With respect to the second point above, the viral TAR sequence is a highly structured region of HIV genomic RNA that binds tightly to viral Tat protein. The Tat:TAR complex is important for efficient generation of viral RNA. Overexpression of the TAR region was envisioned as a decoy molecule that would sequester Tat protein and decrease the

levels of viral RNA. However, TAR proved toxic to most mammalian cells including cells used for manufacturing lentivirus particles. Further, TAR was inefficient for inhibiting viral gene expression in other laboratories and has been discarded as a viable component in HIV gene therapy.

In various embodiments, viral gene sequences have been identified that meet 3 criteria: i) Sequences that are reasonably conserved across a range of HIV isolates representative of the epidemic in a geographic region of interest; ii) reduction in RNA levels due to the activity of an inhibitory RNA in a viral vector will reduce the corresponding protein levels by an amount sufficient to meaningfully reduce HIV replication; and iii) the viral gene sequence(s) targeted by inhibitory RNA are not present in the genes required for packaging and assembling viral vector particles during manufacturing. In various embodiments, a sequence at the junction of HIV Tat and Rev genes and a second sequence within the HIV Vif gene have been targeted by inhibitory RNA. The Tat/Rev targeting has an additional benefit of reducing HIV envelope glycoprotein expression because this region overlaps with the envelope gene in the HIV genome.

Various methods for vector development and testing relies first on identifying suitable targets (as described herein) followed by constructing plasmid DNAs expressing individual or multiple inhibitory RNA species for testing in cell models, and finally constructing lentivirus vectors containing inhibitory RNA with proven anti-HIV function. The lentivirus vectors are tested for toxicity, yield during in vitro production, and effectiveness against HIV in terms of reducing CCR5 expression levels or lowering viral gene products to inhibit virus replication.

Table 2 below demonstrates progression through multiple versions of inhibitory constructs until arriving at a clinical candidate. Initially, shRNA (short homology RNA) molecules were designed and expressed from plasmid DNA constructs.

Plasmids 1-4, as detailed in Table 2 below, tested shRNA sequences against Gag, Pol and RT genes of HIV. While each shRNA was active for suppressing viral protein expression in a cell model, there were two important problems that prevented further development. First, the sequences were targeted to a laboratory isolate of HIV that was not representative of Clade B HIV strains currently circulating in North America and Europe. Second, these shRNA targeted critical components in the lentivirus vector packaging system and would severely reduce vector yield during manufacturing. Plasmid 5, as detailed in Table 2, was selected to target CCR5 and provided a lead candidate sequence. Plasmids 6, 7, 8, 9, 10, and 11, as detailed in Table 2, incorporated the TAR sequence and it was found they produced unacceptable toxicity for mammalian cells including cells used for lentivirus vector manufacturing. Plasmid 2, as detailed in Table 2, identified a lead shRNA sequence capable of reducing Tat RNA expression. Plasmid 12, as detailed in Table 2, demonstrated the effectiveness of shCCR5 expressed as a microRNA (miR) in a lentiviral vector and confirmed it should be in the final product. Plasmid 13, as detailed in Table 2, demonstrated the effectiveness of a shVif expressed as a microRNA (miR) in a lentiviral vector and confirmed it should be in the final product. Plasmid 14, as detailed in Table 2, demonstrated the effectiveness of shTat expressed as a microRNA (miR) in a lentiviral vector and confirmed it should be in the final product. Plasmid 15, as detailed in Table 2, contained the miR CCR5, miR Tat and miR Vif in the form of a miR cluster expressed from a single promoter. These miR do not target critical components in the lentivirus vector packaging system and proved to have negligible toxicity for mammalian cells. The miR within the cluster were equally effective to individual miR that were tested previously, and the overall impact was a substantial reduction in replication of a CCR5-tropic HIV BaL strain.

TABLE 2

Development of HIV Vectors					
	Internal Code	Material	Description	Remarks	Decision
1	SIH-H1-shRT-1,3	Lentiviral vector	shRNA construct for RT of LAI strain	Wrong target, lab virus, no virus test	Abandon
2	SIH-H1-shRT43 (Tat/Rev NL4-3)	Lentiviral vector	H1 promoter shRNA Tat/Rev overlap	Tat protein knock-down >90%	Lead

Vector Construction: For Rev/Tat (RT) shRNA, oligonucleotide sequences containing BamHI and EcoRI restriction sites were synthesized by MWG Operon. Two different Rev/Tat target sequences were tested for their ability to decrease Tat mRNA expression. The RT1,3 target sequence is (5'-ATGGCAGGAAGAAGCGAG-3') (SEQ ID NO: 89) and shRNA sequence is (5'-ATGGCAGGAAGAAGCGAGTTCAAGAGACTCCGCTTCTTCTGCCATTTT-3') (SEQ ID NO: 90). The RT43 sequence is (5'-GCGGAGACAGCGACGAAGAGC-3') (SEQ ID NO: 9) and shRNA sequence is (5'-GCGGAGACAGCGACGAAGAGCTTCAAGAGAGCTTCTCGTCGTCTCCGCTTTT-3') (SEQ ID NO: 10). Oligonucleotide sequences were inserted into the pSIH lentiviral vector (System Biosciences).

Functional test for shRNA against Rev/Tat: The ability of the vector to reduce Tat expression was tested using a luciferase reporter plasmid which contained the Rev/Tat target sequences inserted into the 3'-UTR (untranslated region of the mRNA). Either the shRT1,3 or shRT43 plasmid was co-transfected with the plasmid containing luciferase and the Rev/Tat target sequence. There was a 90% reduction in light emission indicating strong function of the shRT43 shRNA sequence but less than 10% with the shRT1,3 plasmid.

TABLE 2-continued

Development of HIV Vectors					
Internal Code	Material	Description	Remarks	Decision	
<p>Conclusion: The SIH-H1-shRT43 was superior to SIH-H1-shRT-1,3 in terms of reducing mRNA levels in the Luciferase assay system. This indicates potent inhibitory activity of the shRT43 sequence and it was selected as a lead candidate for further development.</p>					
3	SIH-H1-shGag-1	Lentiviral vector	shRNA construct for LAI Gag	Inhibits Gag expression but will inhibit packaging	Abandon
<p>Vector Construction: For Gag shRNA, oligonucleotide sequences containing BamHI and EcoRI restriction sites were synthesized by MWG Operon. A Gag target sequence was tested for their ability to decrease Gag mRNA expression. The Gag target sequence is (5'-GAAGAAATGATGACAGCAT-3') (SEQ ID NO: 11) and shRNA sequence is (5'-GAAGAAATGATGACAGCATTTCAAGAGAATGCTGTCATCTTCTTTT-3') (SEQ ID NO: 12). Oligonucleotide sequences were inserted into the pSIH lentiviral vector (System Biosciences).</p> <p>Functional test for shRNA against Gag: The ability of the vector to reduce Gag expression was tested using a luciferase reporter plasmid which contained the Gag target sequences inserted into the 3'-UTR (untranslated region of the mRNA). The Gag plasmid was co-transfected with the plasmid containing luciferase and the Gag target sequence. There was nearly a 90% reduction in light emission indicating a strong effect of the shGag shRNA sequence.</p> <p>Conclusion: This shRNA sequence is potent against HIV Gag expression but was abandoned. The lentivirus packaging system requires production of Gag from the helper plasmid and shRNA inhibition of Gag will reduce lentivirus vector yield. This shRNA sequence could be used as an oligonucleotide inhibitor of HIV or incorporated into an alternate viral vector packaging system that uses a different vector genome or is modified to resist inhibition by this shRNA.</p>					
4	SIH-H1-shPol-1	Lentiviral vector	shRNA construct for Pol	Inhibits Pol expression but will inhibit packaging	Abandon
<p>Vector Construction: A Pol shRNA was constructed with oligonucleotide sequences containing BamHI and EcoRI restriction sites that were synthesized by MWG Operon. A Pol target sequence was tested for its ability to decrease Pol mRNA expression. The Pol target sequence is (5'-CAGGAGCAGATGATACAG-3') (SEQ ID NO: 13) and shRNA sequence is (5'-CAGGAGATGATACAGTTCAAGAGACTGTATCATCTGCTCCTGTTTT-3') (SEQ ID NO: 14). Oligonucleotide sequences were inserted into the pSIH lentiviral vector (System Biosciences).</p> <p>Functional tests for shRNA against HIV Pol: The ability of the vector to reduce Pol expression was tested using a luciferase reporter plasmid which contained the Pol target sequences inserted into the 3'-UTR (untranslated region of the mRNA). The Pol plasmid was co-transfected with the plasmid containing luciferase and the Pol target sequence. There was a 60% reduction in light emission indicating a strong effect of the shPol shRNA sequence.</p> <p>Conclusion: This shRNA sequence is potent against HIV Pol expression but was abandoned. The lentivirus packaging system requires production of Pol from the helper plasmid and shRNA inhibition of Pol will reduce lentivirus vector yield. This shRNA sequence could be used as an oligonucleotide inhibitor of HIV or incorporated into an alternate viral vector packaging system that uses a different vector genome or is modified to resist inhibition by this shRNA.</p>					
5	SIH-H1-shCCR5-1	Lentiviral vector	shRNA construct for CCR5	Best of 5 candidates, Extracellular CCR5 protein reduction >90%	Lead
<p>Vector Construction: A CCR5 shRNA was constructed with oligonucleotide sequences containing BamHI and EcoRI restriction sites that were synthesized by MWG Operon. Oligonucleotide sequences were inserted into the pSIH lentiviral vector (System Biosciences). The CCR5 target sequence #1, which focuses on CCR5 gene sequence 1 (SEQ ID NO: 25), is (5'-GTGTCAAGTCCAATCTATG-3') (SEQ ID NO: 15) and the shRNA sequence is (5'-GTGTCAAGTCCAATCTATGTTCAAGAGACATAGATTGGACTTGACACTTTTT-3') (SEQ ID NO: 16). The CCR5 target sequence #2, which focuses on CCR5 gene sequence 2 (SEQ ID NO: 26), is (5'-GAGCATGACTGACATCTAC-3') (SEQ ID NO: 17) and the shRNA sequence is (5'-GAGCATGACTGACATCTACTTCAAGAGAGTAGATGTGTCATGCTCTTTTT-3') (SEQ ID NO: 18). The CCR5 target sequence #3, which focuses on CCR5 gene sequence 3 (SEQ ID NO: 27), is (5'-GTAGCTCTAACAGGTTGGA-3') (SEQ ID NO: 19) and the shRNA sequence is (5'-GTAGCTCTAACAGGTTGGATTCAAGAGATCCAACCTGTTAGAGTACTTTTT-3') (SEQ ID NO: 20). The CCR5 target sequence #4, which focuses on CCR5 gene sequence 4 (SEQ ID NO: 28, is (5'-GTTCAAGAACTACTCTTA-3') (SEQ ID NO: 21) and the shRNA sequence is (5'-GTTCAAGAACTACTCTTATTCAAGAGATAAGAGGTAGTTTCTGAAGTTTT-3') (SEQ ID NO: 22). The CCR5 target sequence #5, which focuses on CCR5 gene sequence 5 (SEQ</p>					

TABLE 2-continued

Development of HIV Vectors					
Internal Code	Material	Description	Remarks	Decision	
ID NO: 29), is (5'-GAGCAAGCTCAGTTTACACC-3') (SEQ ID NO: 23) and the shRNA sequence is (5'-GAGCAAGCTCAGTTTACACCTTCAAGAGAGGTGTAACTGAGCTTGCTCTTTTT-3') (SEQ ID NO: 24).					
Functional test for shRNA against CCR5: The ability of a CCR5 shRNA sequence to knock-down CCR5 RNA expression was initially tested by co-transfecting each of the lentiviral plasmids, in separate experiments for each plasmid, containing one of the five CCR5 target sequences with a plasmid expressing the human CCR5 gene. CCR5 mRNA expression was then assessed by qPCR analysis using CCR5-specific primers.					
Conclusion: Based on the reduction in CCR5 mRNA levels the shRNACCR5-1 was most potent for reducing CCR5 gene expression. This shRNA was selected as a lead candidate.					
6	SIH-U6-TAR	Lentiviral vector	U6 promoter-TAR	Toxic to cells	Abandon
7	SIH-U6-TAR-H1-shCCR5	Lentiviral vector	U6 promoter-TAR-H1-shCCR5	Toxic to cells	Abandon
8	U6-TAR-H1-shRT	Lentiviral vector	U6 promoter-TAR-H1-RT	Suppress HIV, toxic to cells, poor packaging	Abandon
9	U6-TAR-75K-shRT	Lentiviral vector	Change shRNA promoter to 7SK	Toxic, poor packaging	Abandon
10	U6-TAR-H1-shRT-H1-shCCR5	Lentiviral vector	U6 promoter-TAR-H1-RT-H1-shCCR5	Toxic, poor packaging, H1 repeats	Abandon
11	U6-TAR-75K-shRT-H1-CCR5	Lentiviral vector	Change shRNA promoter to 7SK	Toxic, poor packaging	Abandon
Vector Construction: A TAR decoy sequence containing flanking KpnI restriction sites was synthesized by MWG operon and inserted into the pSIH lentiviral vector (System Biosciences) at the KpnI site. In this vector, TAR expression is regulated by the U6 promoter. The TAR decoy sequence is (5'-CTTGCAATGATGTCGTAATTGCGTCTTACCTCGTTCTCGACAGCGACCAGATCTGAGCCTGGGAGCTCTCTGGCTGTCTAGTAAAGCTGGTACAGAAAGTTGACGAAAATCTTACTGAGCAAGAAA-3') (SEQ ID NO: 8). Expression of the TAR decoy sequence was determined by qPCR analysis using specific primers for the TAR sequence. Additional vectors were constructed also containing the TAR sequence. The H1 promoter and shRT sequence was inserted in this vector in the XhoI site. The H1 shRT sequence is (5'-GAACGCTGACGTCATCAACCCGCTCCAAGGAATCGCGGGCCAGTGTCACCTAGGCGGGAACACCCAGCGCGCTGCGCCCTGGCAGGAAGATGGCTGTGAGGGACAGGGGAGTGGCGCCCTGCAATATTTGCATGTCGCTATGTGTTCTGGGAAATCACCATAAACGTGAAATGTCTTTGGATTGGGAATCTTATAAGTTCTGTATGAGACCACTTGGATCCGCGGAGACAGCGACGAAGAGCTTCAAGAGAGCTCTTCGTCGTCTCTCCGCTTTTT-3') (SEQ ID NO: 91). This vector could express TAR and knockdown RT. The 7SK promoter was also substituted for the H1 promoter to regulate shRT expression. Another vector was constructed containing U6 TAR, H1 shRT, and H1 shCCR5. The H1 shCCR5 sequence was inserted into the SpeI site of the plasmid containing U6 TAR and H1 shRT. The H1 CCR5 sequence is (5'-GAACGCTGACGTCATCAACCCGCTCCAAGGAATCGCGGGCCAGTGTCACCTAGGCGGGAACACCCAGCGCGCTGCGCCCTGGCAGGAAGATGGCTGTGAGGGACAGGGGAGTGGCGCCCTGCAATATTTGCATGTCGCTATGTGTTCTGGGAAATCACCATAAACGTGAAATGTCTTTGGATTGGGAATCTTATAAGTTCTGTATGAGACCACTTGGATCCGTGTCAAGTCCAATCTATGTTCAAGAGACATAGATTGGACTTGACACTTTTT-3') (SEQ ID NO: 92). The 7SK promoter was also substituted for the H1 promoter to regulate shRT expression.					
Functional test for TAR decoy activity: We tested the effect of SIH-U6-TAR on packaging efficiency. When TAR sequence was included, the yield of vector in the SIH packaging system was reduced substantially.					
Conclusion: Lentivirus vectors expressing the TAR decoy sequence are unsuitable for commercial development due to low vector yields. These constructs were abandoned.					
12	shCCR5	Lentiviral vector	microRNA sequence	Extracellular CCR5 protein reduction >90%	Lead
Vector Construction: A CCR5 microRNA was constructed with oligonucleotide sequences containing BsrGI and NotI restriction sites that were synthesized by MWG Operon. Oligonucleotide sequences were inserted into the pCDH lentiviral vector (System Biosciences). The EF-1 promoter was substituted for a CMV promoter that was used in the plasmid construct Test Material 5. The EF-1 promoter was synthesized by MWG Operon containing flanking ClaI and BsrGI restriction sites and inserted into the pCDH vector containing shCCR5-1. The EF-1 promoter sequence is (5'-					

TABLE 2-continued

Development of HIV Vectors					
Internal Code	Material	Description	Remarks	Decision	
<p>CCGGTGCCTAGAGAAGGTGGCGCGGGGTAACTGGGAAAGTGATGTCGTGACTGGC TCCGCCTTTTCCCGAGGGTGGGGGAGAACCGTATATAAGTGCAGTAGTCGCCGTGA ACGTTCTTTTTCGCAACGGGTTTGCCTCCAGAACACAGGTAAGTCCCGTGTGTGGTTC CCGCGGGCCTGGCCTCTTTACGGGTTATGGCCCTTGCGTGCCTTGAATTACTTCCACG CCCTGGCTGCAGTACGTGATCTTGTATCCCGAGCTTCGGGTTGGAAGTGGGTGGGAG AGTTTCGAGGCCTTGCCTTAAGGAGCCCTTCGCCTCGTGTGAGTTGAGGCCTGGC CTGGGCGCTGGGGCGCGCGCTGCGAATCTGGTGGCACCTTCGCGCCTGTCTCGCTGC TTTCGATAAGTCTCTAGCCATTAAAAATTTTGTAGACCTGTGCGACGCTTTTTTCT GGCAAGATAGTCTTGAATGCGGGCCAAGATCTGCACACTGGTATTTTCGGTTTTTGG GGCCGCGGGCGGCGACGGGGCCCGTGCCTCCAGCGCACATGTTCCGGCGAGGCGGGG CCTGCGAGCGCGGCCACCGAGAATCGGACGGGGGTAGTCTCAAGCTGGCCGGCTGC TCTGGTGCCTGGCCTCGCGCCCGGTGTATCGCCCCCGCTGGGCGGCAAGGCTGGCC CGGTCCGACACAGTTGCGTGAGCGGAAAGATGGCCGCTTCCCGGCCCTGTGTCAGGG AGCTCAAAATGGAGGACGCGCGCTCGGGAGAGCGGGCGGGTGTGATCACCACACA AAGGAAAAGGGCCTTTCGTCTCAGCCGTGCTTCACTGTGACTCCACGGAGTACCGG GCGCCGTCCAGGCACCTCGATTAGTTCTCGAGCTTTTGGAGTACGTCGTTTAGGTT GGGGGAGGGGTTTTATGCGATGGAGTTTCCCACTGAGTGGGTGGAGACTGAAG TTAGGCCAGCTTGGCATTGATGTAATTCTCCTTGAATTTGCCCTTTTGTAGTTTGA TCTTGGTTTCACTCTCAAGCCTCAGACAGTGGTTCAAAGTTTTTCTTCCATTTCAGGT GTCGTGA-3') (SEQ ID NO: 4).</p>					
<p>Functional test for lentivirus CDH-shCCR5-1: The ability of the miR CCR5 sequences to knock-down CCR5 expression was determined by transducing CEM-CCR5 T cells and measuring cell surface CCR5 expression after staining with a fluorescently-labeled monoclonal antibody against CCR5 and measuring the intensity of staining, that is directly proportional to the number of cell surface CCR5 molecules, by analytical flow cytometry. The most effective shRNA sequence for targeting CCR5 was CCR5 shRNA sequence #1. However, the most effective CCR5 targeting sequence for constructing the synthetic microRNA sequence was overlapping with CCR5 sequence #5; this conclusion was based on sequence alignments and experience with miRNA construction. Finally, the miR30 hairpin sequence was used to construct the synthetic miR30 CCR5 sequence which is (5'-AGGTATATTGCTGTGACAGTGAGCGACTGTAACTGAGCTTGCTCTACTGTGAAGCCACAGATGGGTAGAGCAAGCACAGTTTACCGCTGCCTACTGCCTCGGACTTCAAGGGGCTT-3') (SEQ ID NO: 1). The miR CCR5 target sequence is (5'-GAGCAAGCTCAGTTTACA-3') (SEQ ID NO: 5). At multiplicity of infection equal to 5, generating on average 1.25 genome copies of integrated lentivirus per cell, CCR5 expression levels were reduce by ≥90% indicating potent inhibition of CCR5 mRNA by the miR30CCR5 micro RNA construct in a lentivirus vector.</p>					
<p>Conclusion: The miR30CCR5 construct is potent for reducing CCR5 cell surface expression and is a lead candidate for a therapeutic lentivirus for HIV.</p>					
13	shVif	Lentiviral vector	microRNA sequence	Vif protein reduction > 80%	Lead
<p>Vector Construction: A Vif microRNA was constructed with oligonucleotide sequences containing BsrGI and NotI restriction sites that were synthesized by MWG Operon. Oligonucleotide sequences were inserted into the pCDH lentiviral vector (System Biosciences) containing an EF-1 promoter. Based on sequence alignments and experience with constructing synthetic miRNA, the miR21 hairpin sequence was used to construct the synthetic miR21 Vif sequence which is (5'-CATCTCCATGGCTGTACCACCTTGTGCGGGGATGTGTACTTCTGAACCTGTGTTGAATCTCATGGAGTTTCAAGAACACATCCGCACTGACATTTTGGTATCTTTTCATCTGACCA-3') (SEQ ID NO: 2). The miR Vif target sequence is (5'-GGGATGTGTACTTCTGAACCT-3') (SEQ ID NO: 6).</p>					
<p>Functional test for potency of miR21Vif: The ability of the miR Vif sequence to knock-down Vif expression was determined by measuring Vif protein expression by immunoblot analysis using an anti-Vif monoclonal antibody to identify the Vif protein.</p>					
<p>Conclusion: the miR21Vif reduced Vif protein expression by ≥ 10-fold as determined by quantitative image analysis of immunoblot data. This was sufficient to justify miR21Vif as a lead candidate for our therapeutic lentivirus.</p>					
14	shTat	Lentiviral vector	microRNA sequence	Tat RNA reduction > 80%	Lead
<p>Vector Construction: A Tat microRNA was constructed with oligonucleotide sequences containing BsrGI and NotI restriction sites that were synthesized by MWG Operon. The microRNA cluster was inserted into the pCDH lentiviral vector (System Biosciences) containing an EF-1 promoter. Based on sequence alignments and experience in the construction of synthetic miRNA, the miR185 hairpin sequence was selected for constructing a synthetic miR185 Tat sequence which is (5'-GGCCTGGCTCGAGCAGGGGCGAGGGATTCCGCTTCTTCTGCCATAGCGTGGTCCCCTCCCTATGCGAGCGAGAAGCGGCACCTTCCCTCCCAATGACCGCGTCTTCGTGCG-3'). The miR Tat target sequence is (5'-TCCGCTTCTTCTGCCATAG-3') (SEQ ID NO: 3).</p>					

TABLE 2-continued

Development of HIV Vectors					
Internal Code	Material	Description	Remarks	Decision	
<p>Functional test for potency of miR185Tat: The ability of miR Tat to knock-down Tat expression was determined by measuring Tat mRNA expression by RT-PCR analysis using Tat specific primers. We compared the miR185Tat with a similar miR155Tat on the basis of reducing the relative levels of Tat mRNA.</p> <p>Conclusion: The miR185Tat was approximately twice as potent for reducing Tat mRNA compared to miR155Tat, and was selected as the lead candidate for our therapeutic lentivirus.</p>					
15	shCCR5-shVif-shTat	Lentiviral vector	microRNA cluster sequence	CCR5 reduction < 90%, Vif protein reduction > 80%, Tat RNA reduction > 80%, >95% inhibition of HIV replication	Candidate
<p>Vector Construction: A miR30CCR5 miR21Vif miR185Tat microRNA cluster sequence was constructed with a synthetic DNA fragment containing BsrGI and NotI restriction sites that was synthesized by MWG Operon. The DNA fragment was inserted into the pCDH lentiviral vector (System Biosciences) containing the EF-1 promoter. The miR cluster sequence is (5'-AGGTATATTGCTGTTGACAGTGAGCGACTGTAACTGAGCTTGCTCTACTGTGAAGCCACAGATGGGTAGAGCAAGCACAGTTTACCGCTGCCTACTGCCTCGGACTTCAAGGGGCTTCCCGGGCATCTCCATGGCTGTACCACCTTGTGCGGGGATGTGTACTTCTGAAGTTGTGTTGAATCTCATGGAGTTTCAAGAAGACATCCGCACTGACATTTTGGTATCTTTCATCTGACAGCTAGCGGGCCTGGCTCGAGCAGGGGGCGAGGGATTCCGCTTCTTCTGCCATAGCGTGGTCCCTCCCTATGGCAGGCAGAGCGGCACCTTCCCTCCCAATGACCGGTCTTCGTC-3') (SEQ ID NO: 31) and incorporates Test Material 12, Test Material 13 and Test Material 14 into a single cluster that can be expressed under control of the EF-1 promoter.</p> <p>Functional test for potency of the Lentivirus Vector AGT103 containing the microRNA cluster of miR30CCR5, miR21Vif and miR185Tat: The AGT103 vector was tested for potency against CCR5 using the assay for reduction in cell surface CCR5 expression (Test Material 12). The AGT103 vector was tested for potency against Vif using the assay for reduction in cell surface Vif expression (Test Material 13). The AGT103 vector was tested for potency against Tat using the assay for reduction in cell surface Tat expression (Test Material 14).</p> <p>Conclusion: Potency for reducing CCR5 expression by the miRNA cluster was similar to potency observed for the miR30CCR5 alone. Potency for reducing Vif expression by the miRNA cluster was similar to potency observed for the miR21Vif alone. Potency for reducing Tat expression by the miRNA cluster was similar to potency observed for the miR185Tat alone. The miRNA cluster is potent for reducing cell surface CCR5 levels and for inhibiting two HIV genes. Thus, AGT103 containing this miRNA cluster was selected as the therapeutic vector construct for our HIV functional cure program.</p>					

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Functional Assays.

Individual lentivirus vectors containing CCR5, Tat or Vif shRNA sequences and, for experimental purposes, expressing green fluorescent protein (GFP) under control of the CMV Immediate Early Promoter, and designated AGT103/CMV-GFP were tested for their ability to knockdown CCR5, Tat or Vif expression. Mammalian cells were transduced with lentiviral particles either in the presence or absence of polybrene. Cells were collected after 2-4 days; protein and RNA were analyzed for CCR5, Tat or Vif expression. Protein levels were tested by Western blot assay or by labeling cells with specific fluorescent antibodies (CCR5 assay), followed by analytical flow cytometry comparing modified and unmodified cell fluorescence using either the CCR5-specific or isotype control antibodies.

Starting Testing of Lentivirus.

T cell culture medium was made using RPMI 1640 supplemented with 10% FBS and 1% penicillin-streptomycin. Cytokine stocks of IL2 10,000 units/ml, IL-12 1 µg/ml, IL-7 1 µg/ml, IL-15 1 µg/ml were also prepared in advance.

Prior to transduction with the lentivirus, an infectious viral titer was determined and used to calculate the amount of virus to add for the proper multiplicity of infection (MOI).

Day 0-12: Antigen-Specific Enrichment.

On day 0, cryopreserved PBMC were thawed, washed with 10 ml 37° C. medium at 1200 rpm for 10 minutes and resuspended at a concentration of 2×10⁶/ml in 37° C. medium. The cells were cultured at 0.5 ml/well in a 24-well plate at 37° C. in 5% CO₂. To define the optimal stimulation conditions, cells were stimulated with combinations of reagents as listed in Table 3 below:

TABLE 3

1	2	3	4	5	6
IL-2 + IL-12	IL-7 + IL-15	Peptides + IL-2 + IL-12	Peptides + IL-7 + IL-15	MVA + IL-2 + IL-12	MVA + IL-7 + IL-15

50

55

60

65

Final concentrations: IL-2=20 units/ml, IL-12=10 ng/ml, IL-7=10 ng/ml, IL-15=10 ng/ml, peptides=5 µg/ml individual peptide, MVA MOI=1.

On days 4 and 8, 0.5 ml fresh medium and cytokine at listed concentrations (all concentrations indicate the final concentration in the culture) were added to the stimulated cells.

Day 12-24: Non-Specific Expansion and Lentivirus Transduction.

On day 12, the stimulated cells were removed from the plate by pipetting and resuspended in fresh T cell culture medium at a concentration of 1×10^6 /ml. The resuspended cells were transferred to T25 culture flasks and stimulated with DYNABEADS® Human T-Activator CD3/CD28 following the manufacturer's instruction plus cytokine as listed above; flasks were incubated in the vertical position.

On day 14, AGT103/CMV-GFP was added at MOI 20 and cultures were returned to the incubator for 2 days. At this time, cells were recovered by pipetting, collected by centrifugation at 1300 rpm for 10 minutes, resuspended in the same volume of fresh medium, and centrifuged again to form a loose cell pellet. That cell pellet was resuspended in fresh medium with the same cytokines used in previous steps, with cells at 0.5×10^6 viable cells per ml.

From days 14 to 23, the number of the cells was evaluated every 2 days and the cells were diluted to 0.5×10^6 /ml with fresh media. Cytokines were added every time.

On day 24, the cells were collected and the beads were removed from the cells. To remove the beads, cells were transferred to a suitable tube that was placed in the sorting magnet for 2 minutes. Supernatant containing the cells was transferred to a new tube. Cells were then cultured for 1 day in fresh medium at 1×10^6 /ml. Assays were performed to determine the frequencies of antigen-specific T cells and lentivirus transduced cells.

To prevent possible viral outgrowth, amprenavir (0.5 ng/ml) was added to the cultures on the first day of stimulation and every other day during the culture.

Examine Antigen-Specific T Cells by Intracellular Cytokine Staining for IFN-Gamma.

Cultured cells after peptide stimulation or after lentivirus transduction at 1×10^6 cells/ml were stimulated with medium alone (negative control), Gag peptides (5 μ g/ml individual peptide), or PHA (5 μ g/ml, positive control). After 4 hours, BD GolgiPlug™ (1:1000, BD Biosciences) was added to block Golgi transport. After 8 hours, cells were washed and stained with extracellular (CD3, CD4 or CD8; BD Biosciences) and intracellular (IFN-gamma; BD Biosciences) antibodies with BD Cytotfix/Cytoperm™ kit following the manufacturer's instruction. Samples were analyzed on a BD FACSCalibur™ Flow Cytometer. Control samples labeled with appropriate isotype-matched antibodies were included in each experiment. Data were analyzed using Flowjo software.

Lentivirus transduction rate was determined by the frequency of GFP+ cells. The transduced antigen-specific T cells are determined by the frequency of CD3+CD4+GFP+ IFN gamma+ cells; tests for CD3+CD8+GFP+IFN gamma+ cells are included as a control.

These results indicate that CD4 T cells, the target T cell population, can be transduced with lentiviruses that are designed to specifically knock down the expression of HIV-specific proteins, thus producing an expandable population of T cells that are immune to the virus. This example serves as a proof of concept indicating that the disclosed lentiviral constructs can be used in combination with vaccination to produce a functional cure in HIV patients.

Example 4: CCR5 Knockdown with Experimental Vectors

AGTc120 is a HeLa cell line that stably expresses large amounts of CD4 and CCR5. AGTc120 was transduced with

or without LV-CMV-mCherry (the red fluorescent protein mCherry expressed under control of the CMV Immediate Early Promoter) or AGT103/CMV-mCherry. Gene expression of the mCherry fluorescent protein was controlled by a CMV (cytomegalovirus immediate early promoter) expression cassette. The LV-CMV-mCherry vector lacked a microRNA cluster, while AGT103/CMV-mCherry expressed therapeutic miRNA against CCR5, Vif, and Tat.

As shown in FIG. 8A, transduction efficiency was >90%. After 7 days, cells were collected and stained with fluorescent monoclonal antibody against CCR5 and subjected to analytical flow cytometry. Isotype controls are shown in gray on these histograms plotting Mean Fluorescence Intensity of CCR5 APC (x axis) versus cell number normalized to mode (y axis). After staining for cell surface CCR5, cells treated with no lentivirus or control lentivirus (expressing only the mCherry marker) showed no changes in CCR5 density while AGT103 (right section) reduced CCR5 staining intensity to nearly the levels of isotype control. After 7 days, cells were infected with or without R5-tropic HIV reporter virus Bal-GFP. 3 days later, cells were collected and analyzed by flow cytometry. More than 90% of cells were transduced. AGT103-CMV/CMVmCherry reduced CCR5 expression in transduced AGTc120 cells and blocked R5-tropic HIV infection compared with cells treated with the Control vector.

FIG. 8B shows the relative insensitivity of transduced AGTc120 cells to infection with HIV. As above, the lentivirus vectors express mCherry protein and a transduced cell that was also infected with HIV (expressing GFP) would appear as a double positive cell in the upper right quadrant of the false color flow cytometry dot plots. In the absence of HIV (upper panels), there were no GFP+ cells under any condition. After HIV infection (lower panels), 56% of cells were infected in the absence of lentivirus transduction and 53.6% of cells became infected in AGTc120 cells transduced with the LV-CMV-mCherry. When cells were transduced with the therapeutic AGT103/CMV-mCherry vector, only 0.83% of cells appeared in the double positive quadrant indicating they were transduced and infected.

Dividing 53.62 (proportion of double positive cells with control vector) by 0.83 (the proportion of double positive cells with the therapeutic vector) shows that AGT103 provided greater than 65-fold protection against HIV in this experimental system.

Example 5: Regulation of CCR5 Expression by shRNA Inhibitor Sequences in a Lentiviral Vector

Inhibitory RNA Design.

The sequence of *Homo sapiens* chemokine receptor CCR5 (CCR5, NC 000003.12) was used to search for potential siRNA or shRNA candidates to knockdown CCR5 levels in human cells. Potential RNA interference sequences were chosen from candidates selected by siRNA or shRNA design programs such as from the Broad Institute or the BLOCK-IT RNA iDesigner from Thermo Scientific. A shRNA sequence may be inserted into a plasmid immediately after a RNA polymerase III promoter such as H1, U6, or 7SK to regulate shRNA expression. The shRNA sequence may also be inserted into a lentiviral vector using similar promoters or embedded within a microRNA backbone to allow for expression by an RNA polymerase II promoter such as CMV or EF-1 alpha. The RNA sequence may also be synthesized as a siRNA oligonucleotide and utilized independently of a plasmid or lentiviral vector.

Plasmid Construction.

For CCR5 shRNA, oligonucleotide sequences containing BamHI and EcoRI restriction sites were synthesized by MWG Operon. Oligonucleotide sequences were annealed by incubating at 70° C. then cooled to room temperature. Annealed oligonucleotides were digested with the restriction enzymes BamHI and EcoRI for one hour at 37° C., then the enzymes were inactivated at 70° C. for 20 minutes. In parallel, plasmid DNA was digested with the restriction enzymes BamHI and EcoRI for one hour at 37° C. The digested plasmid DNA was purified by agarose gel electrophoresis and extracted from the gel using a DNA gel extraction kit from Invitrogen. The DNA concentration was determined and the plasmid to oligonucleotide sequence was ligated in the ratio 3:1 insert to vector. The ligation reaction was done with T4 DNA ligase for 30 minutes at room temperature. 2.5 µL of the ligation mix were added to 25 µL of STBL3 competent bacterial cells. Transformation required heat shock at 42° C. Bacterial cells were spread on agar plates containing ampicillin and colonies were expanded in L broth. To check for insertion of the oligo sequences, plasmid DNA was extracted from harvested bacterial cultures using the Invitrogen DNA Miniprep kit and tested by restriction enzyme digestion. Insertion of the shRNA sequence into the plasmid was verified by DNA sequencing using a primer specific for the promoter used to regulate shRNA expression.

Functional Assay for CCR5 mRNA Reduction:

The assay for inhibition of CCR5 expression required co-transfection of two plasmids. The first plasmid contains one of five different shRNA sequences directed against CCR5 mRNA. The second plasmid contains the cDNA sequence for human CCR5 gene. Plasmids were co-transfected into 293T cells. After 48 hours, cells were lysed and RNA was extracted using the RNeasy kit from Qiagen. cDNA was synthesized from RNA using a Super Script Kit from Invitrogen. The samples were then analyzed by quantitative RT-PCR using an Applied Biosystems Step One PCR machine. CCR5 expression was detected with SYBR Green from Invitrogen using the forward primer (5'-AGGAATTGATGGCGAGAAGG-3') (SEQ ID NO: 93) and reverse primer (5'-CCCCAAGAAGGTCAAGGTAATCA-3') (SEQ ID NO: 94) with standard conditions for polymerase chain reaction analysis. The samples were normalized to the mRNA for beta actin gene expression using the forward primer (5'-AGCGCGCTACAGCTTCA-3') (SEQ ID NO: 95) and reverse primer (5'-GGCGACGTAGCACAGCTTCP-3') (SEQ ID NO: 96) with standard conditions for polymerase chain reaction analysis. The relative expression of CCR5 mRNA was determined by its Ct value normalized to the level of actin messenger RNA for each sample. The results are shown in FIGS. 9A-9B.

As shown in FIG. 9A, CCR5 knock-down was tested in 293T cells by co-transfection of the CCR5 shRNA construct and a CCR5-expressing plasmid. Control samples were transfected with a scrambled shRNA sequence that did not target any human gene and the CCR5-expressing plasmid. After 60 hours post-transfection, samples were harvested and CCR5 mRNA levels were measured by quantitative PCR. Further, as shown in FIG. 9B, CCR5 knock-down after transduction with lentivirus expressing CCR5 shRNA-1 (SEQ ID NO: 16).

Example 6: Regulation of HIV Components by shRNA Inhibitor Sequences in a Lentiviral Vector

Inhibitory RNA Design.

The sequences of HIV type 1 Rev/Tat (5'-GCGGA-GACAGCGACGAAGAGC-3') (SEQ ID NO: 9) and Gag (5'-GAAGAAATGATGACAGCAT-3') (SEQ ID NO: 11) were used to design: Rev/Tat: (5'-GCGGAGACAGCGAC-GAAGAGCTTCAAGAGAGCTCTTCGTCGCTGTCTC-CGCTTTT-3') (SEQ ID NO: 10) and Gag: (5'-GAAGAAATGATGACAGCATTTCAAGAGAATGCT-GTCATCATTCTTCTTTT-3') (SEQ ID NO: 12) shRNA that were synthesized and cloned into plasmids as described above.

Plasmid Construction.

The Rev/Tat or Gag target sequences were inserted into the 3'UTR (untranslated region) of the firefly luciferase gene used commonly as a reporter of gene expression in cells or tissues. Additionally, one plasmid was constructed to express the Rev/Tat shRNA and a second plasmid was constructed to express the Gag shRNA. Plasmid constructions were as described above.

Functional Assay for shRNA Targeting of Rev/Tat or Gag mRNA:

Using plasmid co-transfection we tested whether a shRNA plasmid was capable of degrading luciferase messenger RNA and decreasing the intensity of light emission in co-transfected cells. A shRNA control (scrambled sequence) was used to establish the maximum yield of light from luciferase transfected cells. When the luciferase construct containing a Rev/Tat target sequence inserted into the 3'-UTR (untranslated region of the mRNA) was co-transfected with the Rev/Tat shRNA sequence there was nearly a 90% reduction in light emission indicating strong function of the shRNA sequence. A similar result was obtained when a luciferase construct containing a Gag target sequence in the 3'-UTR was co-transfected with the Gag shRNA sequence. These results indicate potent activity of the shRNA sequences.

As shown in FIG. 10A, knock-down of the Rev/Tat target gene was measured by a reduction of luciferase activity, which was fused with the target mRNA sequence in the 3'UTR, by transient transfection in 293T cells. As shown in FIG. 10B, knock-down of the Gag target gene sequence fused with the luciferase gene. The results are displayed as the mean±SD of three independent transfection experiments, each in triplicate.

Example 7: AGT103 Decreases Expression of Tat and Vif

Cells were transfected with exemplary vector AGT103/CMV-GFP. AGT103 and other exemplary vectors are defined in Table 3 below.

TABLE 3

Vector Designation	Composition
AGT103	EF1-miR30CCR5-miR21Vif-miR185-Tat-WPRE
Control-mCherry	CMV-mCherry
AGT103/CMV-mCherry	CMV-mCherry-EF1-miR30CCR5-miR21Vif-miR185-Tat-WPRE
Control-GFP	CMV-mCherry

TABLE 3-continued

Vector Designation	Composition
AGT103/CMV-GFP	CMV-GFP-EF1-miR30CCR5-miR21Vif-miR185-Tat-WPRE-

Abbreviations:

EF-1: elongation factor 1 transcriptional promoter

miR30CCR5 - synthetic microRNA capable of reducing CCR5 protein on cell surfaces

miR21Vif - synthetic microRNA capable of reducing levels of HIV RNA and Vif protein expression

miR185Tat - synthetic micro RNA capable of reducing levels of HIV RNA and Tat protein expression

CMV - Immediate early transcriptional promoter from human cytomegalovirus

mCherry - coding region for the mCherry red fluorescent protein

GFP - coding region for the green fluorescent protein

WPRE - Woodchuck hepatitis virus post transcriptional regulatory element

A T lymphoblastoid cell line (CEM; CCRF-CEM; American Type Culture Collection Catalogue number CCL119) was transduced with AGT103/CMV-GFP. 48 hours later the cells were transfected with an HIV expression plasmid encoding the entire viral sequence. After 24 hours, RNA was extracted from cells and tested for levels of intact Tat sequences using reverse transcriptase polymerase chain reaction. Relative expression levels for intact Tat RNA were reduced from approximately 850 in the presence of control lentivirus vector, to approximately 200 in the presence of AGT103/CMV-GFP for a total reduction of >4 fold, as shown in FIG. 11.

Example 8: Regulation of HIV Components by Synthetic MicroRNA Sequences in a Lentiviral Vector

Inhibitory RNA Design.

The sequence of HIV-1 Tat and Vif genes were used to search for potential siRNA or shRNA candidates to knock-down Tat or Vif levels in human cells. Potential RNA interference sequences were chosen from candidates selected by siRNA or shRNA design programs such as from the Broad Institute or the BLOCK-IT RNA iDesigner from Thermo Scientific. The selected shRNA sequences most potent for Tat or Vif knockdown were embedded within a microRNA backbone to allow for expression by an RNA polymerase II promoter such as CMV or EF-1 alpha. The RNA sequence may also be synthesized as a siRNA oligonucleotide and used independently of a plasmid or lentiviral vector.

Plasmid Construction.

The Tat target sequence (5'-TCCGCTTCTTCCTGC-CATAG-3') (SEQ ID NO: 7) was incorporated into the miR185 backbone to create a Tat miRNA (5'-GGGCCTG-GCTCGAGCAGGGGCGAGGGATTCCGCTTCTTCCTGCCATAGCGTGGTCCC CTCCCCTATGGCAGGCA-GAAGCGGCACCTTCCCTCCCAATGACCGCGTCTTCGTCCG-3') (SEQ ID NO: 3) that was inserted into a lentivirus vector and expressed under control of the EF-1 alpha promoter. Similarly, the Vif target sequence (5'-GGGATGT-GTACTTCTGAACCTT-3') (SEQ ID NO: 6) was incorporated into the miR21 backbone to create a Vif miRNA (5'-CATCTCCATGGCTGTACACCTTGTCTGGGGGATGTGTACTTCTGAACCTTGTGTTGAAT CTCATGGAGTTCAGAAGAACACATCCGCACTGACATTTTGG-TATCTTTCATCTGACCA-3') (SEQ ID NO: 2) that was inserted into a lentivirus vector and expressed under control of the EF-1 alpha promoter. The resulting Vif/Tat miRNA-expressing lentivirus vectors were produced in 293T cells using a lentiviral vector packaging system. The Vif and Tat miRNA were embedded into a microRNA cluster consisting

of miR CCR5, miR Vif, and miR Tat all expressed under control of the EF-1 promoter.

Functional Assay for miR185Tat Inhibition of Tat mRNA Accumulation.

A lentivirus vector expressing miR185 Tat (LV-EF 1-miR-CCR5-Vif-Tat) was used at a multiplicity of infection equal to 5 for transducing 293T cells. 24 hours after transduction the cells were transfected with a plasmid expressing HIV strain NL4-3 (pNL4-3) using Lipofectamine2000 under standard conditions. 24 hours later RNA was extracted and levels of Tat messenger RNA were tested by RT-PCR using Tat-specific primers and compared to actin mRNA levels for a control.

Functional Assay for miR21 Vif Inhibition of Vif Protein Accumulation.

A lentivirus vector expressing miR21 Vif (LV-EF1-miR-CCR5-Vif-Tat) was used at a multiplicity of infection equal to 5 for transducing 293T cells. 24 hours after transduction, the cells were transfected with a plasmid expressing HIV strain NL4-3 (pNL4-3) using Lipofectamine2000. 24 hours later cells were lysed and total soluble protein was tested to measure the content of Vif protein. Cell lysates were separated by SDS-PAGE according to established techniques. The separated proteins were transferred to nylon membranes and probed with a Vif-specific monoclonal antibody or actin control antibody.

As shown in FIG. 12A, Tat knock-down was tested in 293T cells transduced with either a control lentiviral vector or a lentiviral vector expressing either synthetic miR185 Tat or miR155 Tat microRNA. After 24 hours, the HIV vector pNL4-3 was transfected with Lipofectamine2000 for 24 hours and then RNA was extracted for qPCR analysis with primers for Tat. As shown in FIG. 12B, Vif knock-down was tested in 293T cells transduced with either a control lentiviral vector or a lentiviral vector expressing a synthetic miR21 Vif microRNA. After 24 hours, the HIV vector pNL4-3 was transfected with Lipofectamine2000 for 24 hours and then protein was extracted for immunoblot analysis with an antibody for HIV Vif.

Example 9: Regulation of CCR5 Expression by Synthetic MicroRNA Sequences in a Lentiviral Vector

CEM-CCR5 cells were transduced with a lentiviral vector containing a synthetic miR30 sequence for CCR5 (AGT103: TGTAAGCTGAGCTTGCTCTA (SEQ ID NO: 97), AGT103-R5-1: TGTAAGCTGAGCTTGCTCGC (SEQ ID NO: 98), or AGT103-R5-2: CATAGATTGGACTTGACAC (SEQ ID NO: 99). After 6 days, CCR5 expression was determined by FACS analysis with an APC-conjugated CCR5 antibody and quantified by mean fluorescence intensity (MFI). CCR5 levels were expressed as % CCR5 with LV-Control set at 100%. The target sequence of AGT103 and AGT103-R5-1 is in the same region as CCR5 target sequence #5. The target sequence of AGT103-R5-2 is the same as CCR5 target sequence #1. AGT103 (2% of total CCR5) is most effective at reducing CCR5 levels as compared with AGT103-R5-1 (39% of total CCR5) and AGT103-R5-2 which does not reduce CCR5 levels. The data is demonstrated in FIG. 13 herein.

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Example 10: Regulation of CCR5 Expression by
Synthetic MicroRNA Sequences in a Lentiviral
Vector Containing Either a Long or Short WPRE
Sequence

Vector Construction.

Lentivirus vectors often require an RNA regulatory element for optimal expression of therapeutic genes or genetic constructs. A common choice is to use the Woodchuck hepatitis virus post transcriptional regulatory element (WPRE). We compared AGT103 that contains a full-length WPRE:

(SEQ ID NO: 32)
(5' AATCAACCTCTGATTACAAAATTTGTGAAAGATTGACTGGTATTCTT
AACTATGTTGCTCCTTTACGCTATGTGGATACGCTGCTTAATGCCTTT
GTATCATGCTATTGCTTCCCGTATGGCTTTCATTTCTCCTCCTTGTATA
AATCCTGGTTGCTGTCTCTTTATGAGGAGTTGTGGCCCGTTGTGAGGCAA
CGTGGCGTGGTGTGCACTGTGTTTGTGACGCAACCCCACTGGTTGGGG
CATTGCCACCACTGTGAGCTCCTTTCCGGGACTTTCGCTTTCCCCCTCC
CTATTGCCACGGCGGAACATCATCGCCGCTGCCTTGCCCGCTGCTGGACA
GGGGCTCGGCTGTTGGGCACTGACAATCCGTGGTGTGTCGGGGAAATC
ATCGTCTCTTCTGGCTGCTCGCTGTGTTGCCACCTGGATTCTGCGCG
GGACGTCCTTCTGCTACGTCCTTCCGCCCTCAATCCAGCGGACCTTCCT
TCCCGCGGCTGCTGCCGGCTCTCGCGCTCTTCGCGCTTCGCGCTTCG
CCCTCAGACGAGTCGGATCTCCCTTTGGGCCGCTCCCGCCT-3')

with a modified AGT103 vector containing a shortened WPRE element

(SEQ ID NO: 80)
(5' AATCAACCTCTGGATTACAAAATTTGTGAAAGATTGACTGATATTCT
TAACTATGTTGCTCCTTTTACGCTGTGTGGATATGCTGCTTAATGCCTC
TGTATCATGCTATTGCTTCCCGTACGGCTTTCGTTTCTCCTCCTTGTAT
AAATCCTGGTTGCTGTCTCTTTATGAGGAGTTGTGGCCCGTTGTCCGTCA
ACGTGGCGTGGTGTGCTGTGTTTGTGACGCAACCCCACTGGCTGGG
GCATTGCCACCACTGTCAACTCCTTCTGGGACTTTCGCTTTCCCCCTC
CCGATCGCCACGGCAGAACTCATCGCCGCTGCCTTGCCCGCTGCTGGAC
AGGGGCTAGGTTGCTGGGCACTGATAATTCGCTGGTGTGTC-3').

Functional Assay for Modulating Cell Surface CCR5 Expression as a Function of Long Versus Short WPRE Element in the Vector Sequence.

AGT103 containing long or short WPRE elements were used for transducing CEM-CCR5 T cells a multiplicity of infection equal to 5. Six days after transduction cells were collected and stained with a monoclonal antibody capable of detecting cell surface CCR5 protein. The antibody was conjugated to a fluorescent marker and the intensity of staining is directly proportional to the level of CCR5 on the cell surface. A control lentivirus had no effect on cell surface CCR5 levels resulting in a single population with a mean fluorescence intensity of 73.6 units. The conventional AGT103 with a long WPRE element reduced CCR5 expression to a mean fluorescence intensity level of 11 units.

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AGT103 modified to incorporate a short WPRE element resulted in a single population of cells with mean fluorescence intensity of 13 units. Accordingly, substituting a short WPRE element had little or no effect on the capacity for AGT103 to reduce cell surface CCR5 expression.

As shown in FIG. 14, CEM-CCR5 cells were transduced with AGT103 containing either a long or short WPRE sequence. After 6 days, CCR5 expression was determined by FACS analysis with an APC-conjugated CCR5 antibody and quantified as mean fluorescence intensity (MFI). CCR5 levels were expressed as % CCR5 with LV-Control set at 100%. The reduction in CCR5 levels was similar for AGT103 with either the short (5.5% of total CCR5) or long (2.3% of total CCR5) WPRE sequence.

Example 11: Regulation of CCR5 Expression by
Synthetic MicroRNA Sequences in a Lentiviral
Vector with or without a WPRE Sequence

Vector Construction.

In order to test whether WPRE was required for AGT103 down regulation of CCR5 expression we constructed a modified vector without WPRE element sequences.

Functional assay for modulating cell surface CCR5 expression as a function of including or not including a long WPRE element in the AGT103 vector. In order to test whether WPRE was required for AGT103 modulation of CCR5 expression levels we transduced CEM-CCR5 T cells with AGT103 or a modified vector lacking WPRE using a multiplicity of infection equal to 5. Six days after transduction cells were collected and stained with a monoclonal antibody capable of recognizing cell surface CCR5 protein. The monoclonal antibody was directly conjugated to a fluorescent marker and the intensity of staining is directly proportional to the number of CCR5 molecules per cell surface. A lentivirus control vector had no effect on cell surface CCR5 levels resulting in a uniform population with mean fluorescence intensity of 164. The lentivirus vector (AGT103 with a long WPRE and also expressing GFP marker protein), AGT103 lacking GFP but containing a long WPRE element, or AGT103 lacking both GFP and WPRE all were similarly effective for modulating cell surface CCR5 expression. After removing GFP, AGT103 with or without WPRE elements were indistinguishable in terms of their capacity for modulating cell surface CCR5 expression.

CEM-CCR5 cells were transduced with AGT103 with or without GFP and WPRE. After 6 days, CCR5 expression was determined by FACS analysis with an APC-conjugated CCR5 antibody and quantified as mean fluorescence intensity (MFI). CCR5 levels were expressed as % CCR5 with LV-Control set at 100%. The reduction in CCR5 levels was similar for AGT103 with (0% of total CCR5) or without (0% of total CCR5) the WPRE sequence. This data is demonstrated in FIG. 15.

Example 12: Regulation of CCR5 Expression by a
CD4 Promoter Regulating Synthetic MicroRNA
Sequences in a Lentiviral Vector

Vector Construction.

A modified version of AGT103 was constructed to test the effect of substituting alternate promoters for expressing the microRNA cluster that suppresses CCR5, Vif and Tat gene expression. In place of the normal EF-1 promoter we substituted the T cell-specific promoter for CD4 glycoprotein expression using the sequence:

(SEQ ID NO: 30)
 (5'TGTTGGGGTTCAAATTGAGCCCCAGCTGTTAGCCCTCTGCAAGAA
 AAAAAAAAAAAAAAAAAAGAACAAAGGGCCTAGATTTCCTTCTGAGCCCCA
 CCCTAAGATGAAGCCTCTTCTTTCAAGGGAGTGGGGTTGGGGTGGAGCG
 GATCCTGTGAGCTTTGCTCTCTCTGTGGCTGGCAGTTTCTCAAAGGGTA
 ACAGGTGTGAGCTGGCTGAGCCTAGGCTGAACCCCTGAGACATGCTACCTC
 TGTCTTCTCATGGCTGGAGGCGACCTTTGTAAGTCACAGAAAGTAGCTGA
 GGGGCTCTGGAAGAACAGCCAGGGTGGAGGTAGATTGCTTTGACT
 CCTGATTTAAGCCTGATTCTGCTTAACCTTTTCCCTTGACTTTGGCATT
 TCACTTTGACATGTTCCCTGAGAGCCTGGGGGTGGGAACCCAGCTCCA
 GCTGTGACGTTTGGGGCCGCCAGGCTAGGGTGTGGAGGAGCCTTGC
 CATCGGGCTTCTGTCTCTCTTCAATTAAGCACGACTCTGCAGA-3').

Functional Assay Comparing EF-1 and CD4 Gene Promoters in Terms of Potency for Reducing Cell Surface CCR5 Protein Expression.

AGT103 modified by substituting the CD4 gene promoter for the normal EF-1 promoter was used for transducing CEM-CCR5 T cells. Six days after transduction cells were collected and stained with a monoclonal antibody capable of recognizing cell surface CCR5 protein. The monoclonal antibody was conjugated to a fluorescent marker and staining intensity is directly proportional to the level of cell surface CCR5 protein. A control lentivirus transduction resulted in a population of CEM-CCR5 T cells that were stained with a CCR5-specific monoclonal antibody and produced a mean fluorescence intensity of 81.7 units. The modified AGT103 using a CD4 gene promoter in place of the EF-1 promoter for expressing microRNA showed a broad distribution of staining with a mean fluorescence intensity roughly equal to 17.3 units. Based on this result, the EF-1 promoter is at least similar and likely superior to the CD4 gene promoter for microRNA expression. Depending on the desired target cell population, the EF-1 promoter is universally active in all cell types and the CD4 promoter is only active in T-lymphocytes.

CEM-CCR5 cells were transduced with a lentiviral vector containing a CD4 promoter regulating a synthetic microRNA sequence for CCR5, Vif, and Tat (AGT103). After 6 days, CCR5 expression was determined by FACS analysis with an APC-conjugated CCR5 antibody and quantified as mean fluorescence intensity (MFI). CCR5 levels were expressed as % CCR5 with LV-Control set at 100%. In cells transduced with LV-CD4-AGT103, CCR5 levels were 11% of total CCR5. This is comparable to that observed for LV-AGT103 which contains the EF1 promoter. This data is demonstrated in FIG. 16.

Example 13: Detecting HIV Gag-Specific CD4 T Cells

Cells and Reagents.

Viable frozen peripheral blood mononuclear cells (PBMC) were obtained from a vaccine company. Data were obtained with a representative specimen from an HIV+ individual who was enrolled into an early stage clinical trial (TRIAL REGISTRATION: clinicaltrials.gov NCT01378156) testing a candidate HIV therapeutic vaccine. Two specimens were obtained for the "Before vaccination" and "After vaccination" studies. Cell culture products,

supplements and cytokines were from commercial suppliers. Cells were tested for responses to recombinant Modified Vaccinia Ankara 62B from Geovax Corporation as described in Thompson, M., S. L. Heath, B. Sweeton, K. Williams, P. Cunningham, B. F. Keele, S. Sen, B. E. Palmer, N. Chomont, Y. Xu, R. Basu, M. S. Hellerstein, S. Kwa and H. L. Robinson (2016). "DNA/MVA Vaccination of HIV-1 Infected Participants with Viral Suppression on Antiretroviral Therapy, followed by Treatment Interruption: Elicitation of Immune Responses without Control of Re-Emergent Virus." *PLoS One* 11(10): e0163164. Synthetic peptides representing the entire HIV-1 Gag polyprotein were obtained from GeoVax the HIV (GAG) Ultra peptide sets were obtained from JPT Peptide Technologies GmbH (www.jpt.com), Berlin, Germany. HIV (GAG) Ultra contains 150 peptides each being 15 amino acids in length and overlapping by 11 amino acids. They were chemically synthesized then purified and analyzed by liquid chromatography—mass spectrometry. Collectively these peptides represent major immunogenic regions of the HIV Gag polyprotein and are designed for average coverage of 57.8% among known HIV strains. Peptide sequences are based on the HIV sequence database from the Los Alamos National Laboratory (<http://www.hiv.lanl.gov/content/sequence/NEWALIGN/align.html>). Peptides are provided as dried trifluoroacetate salts, 25 micrograms per peptide, and are dissolved in approximately 40 microliters of DMSO then diluted with PBS to final concentration. Monoclonal antibodies for detecting CD4 and cytoplasmic IFN-gamma were obtained from commercial sources and intracellular staining was done with the BD Pharmingen Intracellular Staining Kit for interferon-gamma. Peptides were resuspended in DMSO and we include a DMSO only control condition.

Functional Assay for Detecting HIV-Specific CD4+ T Cells.

Frozen PBMC were thawed, washed and resuspended in RPMI medium containing 10% fetal bovine serum, supplements and cytokines. Cultured PBMC collected before or after vaccination were treated with DMSO control, MVA GeoVax (multiplicity of infection equal to 1 plaque forming unit per cell), Peptides GeoVax (1 microgram/ml) or HIV (GAG) Ultra peptide mixture (1 microgram/ml) for 20 hours in the presence of Golgi Stop reagent. Cells were collected, washed, fixed, permeabilized and stained with monoclonal antibodies specific for cell surface CD4 or intracellular interferon-gamma. Stained cells were analyzed with a FAC-SCalibur analytical flow cytometer and data were gated on the CD4+ T cell subset. Cells highlighted within boxed regions are double-positive and designated HIV-specific CD4 T cells on the basis of interferon-gamma expression after MVA or peptide stimulation. Numbers within the boxed regions show the percentage of total CD4 that were identified as HIV-specific. We did not detect strong responses to DMSO or MVA. Peptides from GeoVax elicited fewer responding cells compared to HIV (GAG) Ultra peptide mixture from JPT but differences were small and not significant.

As shown in FIG. 17, PBMCs from a HIV-positive patient before or after vaccination were stimulated with DMSO (control), recombinant MVA expressing HIV Gag from GeoVax (MVA GeoVax), Gag peptide from GeoVax (Pep GeoVax, also referred to herein as Gag peptide pool 1) or Gag peptides from JPT (HIV (GAG) Ultra, also referred to herein as Gag peptide pool 2) for 20 hours. IFN γ production was detected by intracellular staining and flow cytometry using standard protocols. Flow cytometry data were gated on CD4 T cells. Numbers captured in boxes are the percent-

age of total CD4 T cells designated "HIV-specific" on the basis of cytokine response to antigen-specific stimulation.

Example 14: HIV-Specific CD4 T Cell Expansion and Lentivirus Transduction

Designing and testing methods for enriching PBMC to increase the proportion of HIV-specific CD4 T cells and transducing these cells with AGT103 to produce the cellular product AGT103T.

The protocol was designed for ex vivo culture of PBMC (peripheral blood mononuclear cells) from HIV-positive patients who had received a therapeutic HIV vaccine. In this example, the therapeutic vaccine consisted of three doses of plasmid DNA expressing HIV Gag, Pol and Env genes followed by two doses of MVA 62-B (modified vaccinia Ankara number 62-B) expressing the same HIV Gag, Pol, and Env genes. The protocol is not specific for a vaccine product and only requires a sufficient level of HIV-specific CD4+ T cells after immunization. Venous blood was collected and PBMC were purified by Ficoll-Paque density gradient centrifugation. Alternately, PBMC or defined cellular fractions can be prepared by positive or negative selection methods using antibody cocktails and fluorescence activated or magnetic bead sorting. The purified PBMC are washed and cultured in standard medium containing supplements, antibiotics and fetal bovine serum. To these cultures, a pool of synthetic peptides was added representing possible T cell epitopes within the HIV Gag polyprotein. Cultures are supplemented by adding cytokines interleukin-2 and interleukin-12 that were selected after testing combinations of interleukin-2 and interleukin-12, interleukin 2 and interleukin-7, interleukin 2 and interleukin-15. Peptide stimulation is followed by a culture interval of approximately 12 days. During the 12 days culture, fresh medium and fresh cytokine supplements were added approximately once every four days.

The peptide stimulation interval is designed to increase the frequency of HIV-specific CD4 T cells in the PBMC culture. These HIV-specific CD4 T cells were activated by prior therapeutic immunization and can be re-stimulated and caused to proliferate by synthetic peptide exposure. Our goal is to achieve greater than or equal to 1% of total CD4 T cells being HIV-specific by end of the peptide stimulation culture period.

On approximately day 12 of culture cells are washed to remove residual materials then stimulated with synthetic beads decorated with antibodies against CD4 T cell surface proteins CD3 and CD28. This well-established method for polyclonal stimulation of T cells will reactivate the cells and make them more susceptible for AGT103 lentivirus transduction. The lentivirus transduction is performed on approximately day 13 of culture and uses a multiplicity of infection between 1 and 5. After transduction cells are washed to remove residual lentivirus vector and cultured in media containing interleukin-2 and interleukin-12 with fresh medium and cytokines added approximately once every four days until approximately day 24 of culture.

Throughout the culture interval the antiretroviral drug Saquinavir is added at a concentration of approximately 100 nM to suppress any possible outgrowth of HIV.

On approximately day 24 of culture cells are harvested, washed, a sample is set aside for potency and release assay, then the remaining cells are suspended in cryopreservation medium before freezing in single aliquots of approximately 1×10^{10} cells per dose that will contain approximately 1×10^8 HIV-specific CD4 T cells that are transduced with AGT103.

Potency of the cell product (AGT103T) is tested in one of two alternate potency assays. Potency assay 1 tests for the average number of genome copies (integrated AGT103 vector sequences) per CD4 T cell. The minimum potency is approximately 0.5 genome copies per CD4 T cell in order to release the product. The assay is performed by positive selection of CD3 positive/CD4 positive T cells using magnetic bead labeled monoclonal antibodies, extracting total cellular DNA and using a quantitative PCR reaction to detect sequences unique to the AGT103 vector. Potency assay 2 tests for the average number of genome copies of integrated AGT103 within the subpopulation of HIV-specific CD4 T cells. This assay is accomplished by first stimulating the PBMC with the pool of synthetic peptides representing HIV Gag protein. Cells are then stained with a specific antibody reagent capable of binding to the CD4 T cell and also capturing secreted interferon-gamma cytokine. The CD4 positive/interferon-gamma positive cells are captured by magnetic bead selection, total cellular DNA is prepared, and the number of genome copies of AGT103 per cell is determined with a quantitative PCR reaction. Release criterion based on potency using Assay 2 require that greater than or equal to 0.5 genome copies per HIV-specific CD4 T-cell are present in the AGT103 cell product.

Functional Test for Enriching and Transducing HIV-Specific CD4 T Cells from PBMC of HIV-Positive Patients that Received a Therapeutic HIV Vaccine.

The impact of therapeutic vaccination on the frequency of HIV-specific CD4 T cells was tested by a peptide stimulation assay (FIG. 14 panel B). Before vaccination the frequency of HIV-specific CD4 T cells was 0.036% in this representative individual. After vaccination, the frequency of HIV-specific CD4 T cells was increased approximately 2-fold to the value of 0.076%. Responding cells (HIV-specific) identified by accumulation of cytoplasmic interferon-gamma, were only detected after specific peptide stimulation.

We also tested whether peptide stimulation to enrich for HIV-specific CD4 T cells followed by AGT103 transduction would reach our goal of generating approximately 1% of total CD4 T cells in culture that were both HIV-specific and transduced by AGT103. In this case, we used an experimental version of AGT103 that expresses green fluorescence protein (see GFP). In FIG. 14, panel C the post-vaccination culture after peptide stimulation (HIV (GAG) Ultra) and AGT103 transduction demonstrated that 1.11% of total CD4 T cells were both HIV-specific (based on expressing interferon-gamma in response to peptide stimulation) and AGT103 transduced (based on expression of GFP).

Several patients from a therapeutic HIV vaccine study were tested to assess the range of responses to peptide stimulation and to begin defining eligibility criteria for entering a gene therapy arm in a future human clinical trial. FIG. 18D shows the frequency of HIV-specific CD4 T cells in 4 vaccine trial participants comparing their pre- and post-vaccination specimens. In three cases the post-vaccination specimens show a value of HIV-specific CD4 T cells that was greater than or equal to 0.076% of total CD4 T cells. The ability to reach this value was not predicted by the pre-vaccination specimens as patient 001-004 and patient 001-006 both started with pre-vaccination values of 0.02% HIV-specific CD4 T cells but one reached an eventual post-vaccination value of 0.12% HIV-specific CD4 T cells while the other individual failed to increase this value after vaccination. The same three patients that responded well to vaccine, in terms of increasing the frequency of HIV-specific CD4 T cells, also showed substantial enrichment of HIV-specific CD4 T cells after peptide stimulation and culture. In

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the three cases shown in FIG. 18E, peptide stimulation and subsequent culture generated samples where 2.07%, 0.72% or 1.54% respectively of total CD4 T cells were HIV-specific. These values indicate that a majority of individuals responding to a therapeutic HIV vaccine will have a sufficiently large ex vivo response to peptide stimulation in order to enable our goal of achieving approximately 1% of total CD4 T cells that are HIV-specific and transduced with AGT103 in the final cell product.

FIG. 18A describes the schedule of treatment. FIG. 18B demonstrates that PBMCs were stimulated with Gag peptide or DMSO control for 20 hours. IFN gamma production was detected by intracellular staining by FACS. CD4⁺ T cells were gated for analysis. FIG. 18C demonstrates CD4⁺ T cells were expanded and transduced with AGT103-GFP using the method as shown in FIG. 18A. Expanded CD4⁺ T cells were rested in fresh medium without any cytokine for 2 days and re-stimulated with Gag peptide or DMSO control for 20 hours. IFN gamma production and GFP expression was detected by FACS. CD4⁺ T cells were gated for analysis. FIG. 18D demonstrates frequency of HIV-specific CD4⁺ T cells (IFN gamma positive, pre- and post-vaccination) were detected from 4 patients. Panel E demonstrates Post-vaccination PBMCs from 4 patients were expanded and HIV-specific CD4⁺ T cells were examined.

Example 15: Dose Response

Vector Construction.

A modified version of AGT103 was constructed to test the dose response for increasing AGT103 and its effects on cell surface CCR5 levels. The AGT103 was modified to include a green fluorescent protein (GFP) expression cassette under control of the CMV promoter. Transduced cells expression the miR30CCR5 miR21Vif miR185Tat micro RNA cluster and emit green light due to expressing GFP.

Functional Assay for Dose Response of Increasing AGT103-GFP and Inhibition of CCR5 Expression.

CEM-CCR5 T cells were transduced with AGT103-GFP using multiplicity of infection per cell from 0 to 5. Transduced cells were stained with a fluorescently conjugated (APC) monoclonal antibody specific for cell surface CCR5. The intensity of staining is proportional to the number of CCR5 molecules per cell surface. The intensity of green fluorescence is proportional to the number of integrated AGT103-GFP copies per cell.

FIG. 19A demonstrates the dose response for increasing AGT103-GFP and its effects on cell surface CCR5 expression. At multiplicity of infection equal to 0.4 only 1.04% of cells are both green (indicating transduction) and showing significantly reduced CCR5 expression. At multiplicity of infection equal to 1 the number of CCR5^{low}, GFP⁺ cells increases to 68.1%/At multiplicity of infection equal to 5 the number of CCR5^{low}, GFP⁺ cells increased to 95.7%. These data are presented in histogram form in FIG. 19B that shows a normally distribution population in terms of CCR5 staining, moving toward lower mean fluorescence intensity with increasing doses of AGT103-GFP. The potency of AGT103-GFP is presented in graphical form in FIG. 19C showing the percentage inhibition of CCR5 expression with increasing doses of AGT103-GFP. At multiplicity of infection equal to 5, there was greater than 99% reduction in CCR5 expression levels.

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Example 16: AGT103 Efficiently Transduces Primary Human CD4⁺ T Cells

Transducing Primary CD4 T Cells with AGT103 Lentivirus Vector.

A modified AGT103 vector containing the green fluorescence protein marker (GFP) was used at multiplicities of infection between 0.2 and 5 for transducing purified, primary human CD4 T cells.

Functional Assay for Transduction Efficiency of AGT103 in Primary Human CD4 T Cells.

CD4 T cells were isolated from human PBMC (HIV-negative donor) using magnetic bead labeled antibodies and standard procedures. The purified CD4 T cells were stimulated ex vivo with CD3/CD28 beads and cultured in media containing interleukin-2 for 1 day before AGT103 transduction. The relationship between lentivirus vector dose (the multiplicity of infection) and transduction efficiency is demonstrated in FIG. 20A showing that multiplicity of infection equal to 0.2 resulted in 9.27% of CD4 positive T cells being transduced by AGT103 and that value was increased to 63.1% of CD4 positive T cells being transduced by AGT103 with a multiplicity of infection equal to 5. In addition to achieving efficient transduction of primary CD4 positive T cells it is also necessary to quantify the number of genome copies per cell. In FIG. 20B total cellular DNA from primary human CD4 T cells transduced at several multiplicities of infection were tested by quantitative PCR to determine the number of genome copies per cell. In a multiplicity of infection equal to 0.2 we measured 0.096 genome copies per cell that was in good agreement with 9.27% GFP positive CD4 T cells in FIG. 20A. Multiplicity of infection equal to 1 generated 0.691 genome copies per cell and multiplicity of infection equal to 5 generated 1.245 genome copies per cell.

As shown in FIGS. 20A-20B, CD4⁺ T cells isolated from PBMC were stimulated with CD3/CD28 beads plus IL-2 for 1 day and transduced with AGT103 at various concentrations. After 2 days, beads were removed and CD4⁺ T cells were collected. As shown in FIG. 20A, frequency of transduced cells (GFP positive) were detected by FACS. As shown in FIG. 20B, the number of vector copies per cell was determined by qPCR. At a multiplicity of infection (MOI) of 5, 63% of CD4⁺ T cells were transduced with an average of 1 vector copy per cell.

Example 17: AGT103 Inhibits HIV Replication in Primary CD4⁺ T Cells

Protecting Primary Human CD4 Positive T Cells from HIV Infection by Transducing Cells with AGT103.

Therapeutic lentivirus AGT103 was used for transducing primary human CD4 positive T cells at multiplicities of infection between 0.2 and 5 per cell. The transduced cells were then challenged with a CXCR4-tropic HIV strain NL4.3 that does not require cell surface CCR5 for penetration. This assay tests the potency of microRNA against Vif and Tat genes of HIV in terms of preventing productive infection in primary CD4 positive T cells, but uses an indirect method to detect the amount of HIV released from infected, primary human CD4 T cells.

Functional Assay for AGT103 Protection Against CXCR4-Tropic HIV Infection of Primary Human CD4 Positive T Cells.

CD4 T cells were isolated from human PBMC (HIV-negative donor) using magnetic bead labeled antibodies and standard procedures. The purified CD4 T cells were stimulated ex vivo with CD3/CD28 beads and cultured in media

containing interleukin-2 for 1 day before AGT103 transduction using multiplicities of infection between 0.2 and 5. Two days after transduction the CD4 positive T cell cultures were challenged with HIV strain NL4.3 that was engineered to express the green fluorescent protein (GFP). The transduced and HIV-exposed primary CD4 T cell cultures were maintained for 7 days before collecting cell-free culture fluids containing HIV. The cell-free culture fluids were used to infect a highly permissive T cell line C8166 for 2 days. The proportion of HIV-infected C8166 cells was determined by flow cytometry detecting GFP fluorescence. With a mock lentivirus infection, the dose of 0.1 multiplicity of infection for NL4.3 HIV resulted in an amount of HIV being released into culture fluids that was capable of establishing productive infection in 15.4% of C8166 T cells. With the dose 0.2 multiplicity of infection for AGT103, this value for HIV infection of C8166 cells is reduced to 5.3% and multiplicity of infection equal to 1 for AGT103 resulted in only 3.19% of C8166 T cells being infected by HIV. C8166 infection was reduced further to 0.62% after AGT103 transduction using a multiplicity of infection equal to 5. There is a clear dose response relationship between the amount of AGT103 used for transduction and the amount of HIV released into the culture medium.

As shown in FIG. 21, CD4⁺ T cells isolated from PBMC were stimulated with CD3/CD28 beads plus IL-2 for 1 day and transduced with AGT103 at various concentrations (MOI). After 2 days, beads were removed and CD4⁺ T cells were infected with 0.1 MOI of HIV NL4.3-GFP. 24 hours later, cells were washed 3 times with PBS and cultured with IL-2 (30 U/ml) for 7 days. At the end of the culture, supernatant was collected to infect the HIV permissive cell line C8166 for 2 days. HIV-infected C8166 cells (GFP positive) were detected by FACS. There was a reduction in viable HIV with an increase in the multiplicity of infection of AGT103 as observed by less infection of C8166 cells MOI 0.2=65.6%, MOI 1=79.3%, and MOI 5=96%.

Example 18: AGT103 Protects Primary Human CD4⁺ T Cells from HIV-Induced Depletion

AGT103 Transduction of Primary Human CD4 T Cells to Protect Against HIV-Mediated Cytopathology and Cell Depletion.

PBMC were obtained from healthy, HIV-negative donors and stimulated with CD3/CD28 beads then cultured for 1 day in medium containing interleukin-2 before AGT103 transduction using multiplicities of infection between 0.2 and 5.

Functional Assay for AGT103 Protection of Primary Human CD4 T Cells Against HIV-Mediated Cytopathology.

AGT103-transduced primary human CD4 T cells were infected with HIV NL 4.3 strain (CXCR4-tropic) that does not require CCR5 for cellular entry. When using the CXCR4-tropic NL 4.3, only the effect of Vif and Tat microRNA on HIV replication is being tested. The dose of HIV NL 4.3 was 0.1 multiplicity of infection. One day after HIV infection, cells were washed to remove residual virus and cultured in medium plus interleukin-2. Cells were collected every three days during a 14-day culture then stained with a monoclonal antibody that was specific for CD4 and directly conjugated to a fluorescent marker to allow measurement of the proportion of CD4 positive T cells in PBMC. Untreated CD4 T cells or CD4 T cells transduced with the control lentivirus vector were highly susceptible to HIV challenge and the proportion of CD4 positive T cells in PBMC fell below 10% by day 14 culture. In contrast, there

was a dose-dependent effect of AGT103 on preventing cell depletion by HIV challenge. With a AGT103 dose of 0.2 multiplicity of infection more than 20% of PBMC were CD4 T cells by day 14 of culture and this value increased to more than 50% of PBMC being CD4 positive T cells by day 14 of culture with a AGT103 dose of multiplicity of infection equal to 5. Again, there is a clear dose response effect of AGT103 on HIV cytopathogenicity in human PBMC.

As shown in FIG. 22, PBMCs were stimulated with CD3/CD28 beads plus IL-2 for 1 day and transduced with AGT103 at various concentrations (MOI). After 2 days, beads were removed and cells were infected with 0.1 MOI of HIV NL4.3. 24 hours later, cells were washed 3 times with PBS and cultured with IL-2 (30 U/ml). Cells were collected every 3 days and the frequency of CD4⁺ T cells were analyzed by FACS. After 14 days of exposure to HIV, there was an 87% reduction in CD4⁺ T cells transduced with LV-Control, a 60% reduction with AGT103 MOI 0.2, a 37% reduction with AGT103 MOI 1, and a 17% reduction with AGT103 MOI 5.

Example 19: Generating a Population of CD4⁺ T Cells Enriched for HIV-Specificity and Transduced with AGT103/CMV-GFP

Therapeutic vaccination against HIV had minimal effect on the distribution of CD4⁺, CD8⁺ and CD4⁺/CD8⁺ T cells. As shown in FIG. 23A, the CD4 T cell population is shown in the upper left quadrant of the analytical flow cytometry dot plots, and changes from 52% to 57% of total T cells after the vaccination series. These are representative data.

Peripheral blood mononuclear cells from a participant in an HIV therapeutic vaccine trial were cultured for 12 days in medium +/-interleukin-2/interleukin-12 or +/-interleukin-7/interleukin-15. Some cultures were stimulated with overlapping peptides representing the entire p55 Gag protein of HIV-1 (HIV (GAG) Ultra peptide mixture) as a source of epitope peptides for T cell stimulation. These peptides are 10-20 amino acids in length and overlap by 20-50% of their length to represent the entire Gag precursor protein (p55) from HIV-1 BaL strain. The composition and sequence of individual peptides can be adjusted to compensate for regional variations in the predominant circulating HIV sequences or when detailed sequence information is available for an individual patient receiving this therapy. At culture end, cells were recovered and stained with anti-CD4 or anti-CD8 monoclonal antibodies and the CD3⁺ population was gated and displayed here. The HIV (GAG) Ultra peptide mixture stimulation for either pre- or post-vaccination samples was similar to the medium control indicating that HIV (GAG) Ultra peptide mixture was not toxic to cells and was not acting as a polyclonal mitogen. The results of this analysis can be found in FIG. 23B.

HIV (GAG) Ultra peptide mixture and interleukin-2/interleukin-12 provided for optimal expansion of antigen-specific CD4 T cells. As shown in the upper panels of FIG. 23C, there was an increase in cytokine (interferon-gamma) secreting cells in post-vaccination specimens exposed to HIV (GAG) Ultra peptide mixture. In the pre-vaccination sample, cytokine secreting cells increased from 0.43 to 0.69% as a result of exposure to antigenic peptides. In contrast, the post-vaccination samples showed an increase of cytokine secreting cells from 0.62 to 1.76% of total CD4 T cells as a result of peptide stimulation. These data demonstrate the strong impact of vaccination on the CD4 T cell responses to HIV antigen.

Finally, AGT103/CMV-GFP transduction of antigen-expanded CD4 T cells produced HIV-specific and HIV-resistant helper CD4 T cells that are needed for infusion into patients as part of a functional cure for HIV (in accordance with other various aspects and embodiments, AGT103 alone is used; for example, clinical embodiments may not include the CMV-GFP segment). The upper panels of FIG. 23C show the results of analyzing the CD4+ T cell population in culture. The x axis of FIG. 23C shows Green Fluorescent Protein (GFP) emission indicating that individual cells were transduced with the AGT103/CMV-GFP. In the post-vaccination samples 1.11% of total CD4 T cells that were both cytokine secreting was recovered, indicating that the cells are responding specifically to HIV antigen, and transduced with AGT103/CMV-GFP. This is the target cell population and the clinical product intended for infusion and functional cure of HIV. With the efficiency of cell expansion during the antigen stimulation and subsequent polyclonal expansion phases of ex vivo culture, 4×10^8 antigen-specific, lentivirus transduced CD4 T cells can be produced. This exceeds the target for cell production by 4-fold and will allow achievement of a count of antigen-specific and HIV-resistant CD4 T cells of approximately 40 cells/microliter of blood or around 5.7% of total circulating CD4 T cells.

Table 4 below shows the results of the ex vivo production of HIV-specific and HIV-resistant CD4 T cells using the disclosed vectors and methods.

TABLE 4

Material/manipulation	Total CD4 T cells	Percentage HIV-specific	Percentage HIV-specific and HIV-resistant
Leukapheresis pack from HIV+ patient	$\sim 7 \times 10^8$	~ 0.12	N/A
Peptide expansion ex vivo	$\sim 8 \times 10^8$	~ 2.4	N/A
Mitogen expansion	$\sim 1.5 \times 10^{10}$	~ 2.4	N/A
Lentivirus transduction	$\sim 1.5 \times 10^{10}$	~ 2.4	~ 1.6

Example 20: Clinical Study for Treatment of HIV

AGT103T is a genetically modified autologous PBMC containing $>5 \times 10^7$ HIV-specific CD4 T cells that are also transduced with AGT103 lentivirus vector.

A Phase I clinical trial will test the safety and feasibility of infusing ex vivo modified autologous CD4 T cells (AGT103T) in adult research participants with confirmed HIV infection, CD4+ T-cell counts >600 cells per mm^3 of blood and stable virus suppression below 200 copies per ml of plasma while on cART. All study participants will continue receiving their standard antiretroviral medications through the Phase I clinical trial. Up to 40 study participants receive two doses by intramuscular injection 8 weeks apart, of recombinant modified vaccinia Ankara (rMVA) expressing HIV Gag, Pol and Env proteins. Seven to 10 days after the second immunization a blood sample is collected for in vitro testing to measure the frequency of CD4+ T-cells that respond to stimulation with a pool of overlapping, synthetic peptides representing the HIV-1 Gag polypeptide. Subjects in the upper half of vaccine responders, based on measuring the frequency of Gag-specific CD4 T cells are enrolled in the gene therapy arm and subjects in the lower half of responders do not continue in the study. We anticipate that the cut-off for higher responders is a HIV-specific CD4+ T cell frequency $\geq 0.065\%$ of total CD4 T cells. Subjects enrolled into

the gene therapy arm of our trial undergo leukapheresis followed by purification of PBMC (using Ficoll density gradient centrifugation or negative selection with antibodies) that are cultured ex vivo and stimulated with HIV Gag peptides plus interleukin-2 and interleukin-12 for 12 days, then stimulated again with beads decorated with CD3/CD28 bispecific antibody. The antiretroviral drug Saquinavir is included at 100 nM to prevent emergence of autologous HIV during ex vivo culture. One day after CD3/CD28 stimulation cells are transduced with AGT103 at multiplicity of infection between 1 and 10. The transduced cells are cultured for an additional 7-14 days during which time they expand by polyclonal proliferation. The culture period is ended by harvesting and washing cells, setting aside aliquots for potency and safety release assays, and resuspending the remaining cells in cryopreservation medium. A single dose is $\leq 1 \times 10^{10}$ autologous PBMC. The potency assay measures the frequency of CD4 T cells that respond to peptide stimulation by expressing interferon-gamma. Other release criteria include the product must include $\geq 0.5 \times 10^7$ HIV-specific CD4 T cells that are also transduced with AGT103. Another release criterion is that the number of AGT103 genome copies per cell must not exceed 3. Five days before infusion with AGT103T subjects receive one dose of busulfuram (or Cytosar) conditioning regimen followed by infusion of $\leq 1 \times 10^{10}$ PBMC containing genetically modified CD4 T cells.

A Phase II study will evaluate efficacy of AGT103T cell therapy. Phase II study participants include individuals enrolled previously in our Phase I study who were judged to have successful and stable engraftment of genetically modified, autologous, HIV-specific CD4 T cells and clinical responses defined as positive changes in parameters monitored as described in efficacy assessments (1.3.). Study participants will be asked to add Maraviroc to their existing regimen of antiretroviral medication. Maraviroc is a CCR5 antagonist that will enhance the effectiveness of genetic therapy directed at reducing CCR5 levels. Once the Maraviroc regimen is in place subjects will be asked to discontinue the previous antiretroviral drug regimen and only maintain Maraviroc monotherapy for 28 days or until plasma viral RNA levels exceed 10,000 per ml on 2 sequential weekly blood draws. Persistently high viremia requires participants to return to their original antiretroviral drug regimen with or without Maraviroc according to recommendations of their HIV care physician.

If participants remain HIV suppressed (below 2,000 vRNA copies per ml of plasma) for >28 days on Maraviroc monotherapy, they will be asked to gradually reduce Maraviroc dosing over a period of 4 weeks followed by intensive monitoring for an additional 28 days. Subjects who maintained HIV suppression with Maraviroc monotherapy are considered to have a functional cure. Subjects who maintain HIV suppression even after Maraviroc withdrawal also have a functional cure. Monthly monitoring for 6 months followed by less intensive monitoring will establish the durability of functional cure.

Patient Selection

Inclusion Criteria.

Aged between 18 and 60 years.

Documented HIV infection prior to study entry.

Must be willing to comply with study-mandated evaluations; including not changing their antiretroviral regimen (unless medically indicated) during the study period.

CD4+ T-cell count >600 cell per millimeter cubed (cells/ mm^3)

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CD4+ T-cell nadir of >400 cells/mm³
 HIV viral load $<1,000$ copies per milliliter (mL)
 Exclusion Criteria.
 Any viral hepatitis
 Acute HIV infection
 HIV viral load $>1,000$ copies/mL
 Active or recent (prior 6 months) AIDS defining complication
 Any change in HIV medications within 12 weeks of entering the study
 Cancer or malignancy that has not been in remission for at least 5 years with the exception of successfully treated basal cell carcinoma of the skin
 Current diagnosis of NYHA grade 3 or 4 congestive heart failure or uncontrolled angina or arrhythmias
 History of bleeding problems
 Use of chronic steroids in past 30 days
 Pregnant or breast feeding
 Active drug or alcohol abuse
 Serious illness in past 30 days
 Currently participating in another clinical trial or any prior gene therapy

Safety Assessments
 Acute infusion reaction
 Post-infusion safety follow-up

Efficacy Assessments—Phase I
 Number and frequency of modified CD4 T cells.
 Durability of modified CD4 T cells.
 In vitro response to Gag peptide restimulation (ICS assay) as a measure of memory T cell function.
 Polyfunctional anti-HIV CD8 T cell responses compare to pre- and post-vaccination time points.
 Frequency of CD4 T cells making doubly spliced HIV mRNA after in vitro stimulation.

Efficacy Assessments—Phase II
 Number and frequency of genetically modified CD4 T cells.
 Maintenance of viral suppression ($<2,000$ vRNA copies per ml but 2 consecutive weekly draws not exceeding 5×10^4 vRNA copies per ml are permitted) with Maraviroc monotherapy.
 Continued virus suppression during and after Maraviroc withdrawal.
 Stable CD4 T cell count.
 AGT103T Consists of Up to 1×10^{10} Genetically Modified, Autologous CD4+ T Cells Containing $\geq 5 \times 10^7$ HIV-Specific CD4 T Cells that are Also Transduced with AGT103 Lentivirus Vector.

A Phase I clinical trial will test the safety and feasibility of infusing ex vivo modified autologous CD4 T cells (AGT103T) in adult research participants with confirmed HIV infection, CD4+ T-cell counts >600 cells per mm³ of blood and stable virus suppression below 200 copies per ml of plasma while on cART. Up to 40 study participants receive two doses by intramuscular injection 8 weeks apart, of recombinant modified vaccinia Ankara (rMVA) expressing HIV Gag, Pol and Env proteins. Seven to 10 days after the second immunization a blood sample is collected for in vitro testing to measure the frequency of CD4+ T-cells that respond to stimulation with a pool of overlapping, synthetic peptides representing the HIV-1 Gag polyprotein. Subjects in the upper half of vaccine responders, based on measuring the frequency of Gag-specific CD4 T cells are enrolled in the gene therapy arm and subjects in the lower half of responders do not continue in the study. We anticipate that the cut-off

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for higher responders is a HIV-specific CD4+ T cell frequency $>0.065\%$ of total CD4 T cells. Subjects enrolled into the gene therapy arm of our trial undergo leukapheresis and the CD4+ T cells are enriched by negative selection. The enriched CD4 subset is admixed with 10% the number of cells from the CD4-negative subset to provide a source and antigen-presenting cells. The enriched CD4 T cells are stimulated with HIV Gag peptides plus interleukin-2 and interleukin-12 for 12 days, then stimulated again with beads decorated with CD3/CD28 bispecific antibody. The antiretroviral drug Saquinavir is included at 100 nM to prevent emergence of autologous HIV during ex vivo culture. One day after CD3/CD28 stimulation cells are transduced with AGT103 at multiplicity of infection between 1 and 10. The transduced cells are cultured for an additional 7-14 days during which time they expand by polyclonal proliferation. The culture period is ended by harvesting and washing cells, setting aside aliquots for potency and safety release assays, and resuspending the remaining cells in cryopreservation medium. A single dose is $\leq 1 \times 10^{10}$ autologous cells enriched for the CD4+ T cell subset. The potency assay measures the frequency of CD4 T cells that respond to peptide stimulation by expressing interferon-gamma. Other release criteria include that the product must include $\geq 0.5 \times 10^7$ HIV-specific CD4 T cells that are also transduced with AGT103. Another release criterion is that the number of AGT103 genome copies per cell must not exceed 3. Five days before infusion with AGT103T subjects receive one dose of busulfuram (or Cytoxan) conditioning regimen followed by infusion of $\leq 1 \times 10^{10}$ enriched and genetically modified CD4 T cell.

A Phase II study will evaluate efficacy of AGT103T cell therapy. Phase II study participants include individuals enrolled previously in our Phase I study who were judged to have successful and stable engraftment of genetically modified, autologous, HIV-specific CD4 T cells and clinical responses defined as positive changes in parameters monitored as described in efficacy assessments (1.3.). Study participants will be asked to add Maraviroc to their existing regimen of antiretroviral medication. Maraviroc is a CCR5 antagonist that will enhance the effectiveness of genetic therapy directed at reducing CCR5 levels. Once the Maraviroc regimen is in place subjects will be asked to discontinue the previous antiretroviral drug regimen and only maintain Maraviroc monotherapy for 28 days or until plasma viral RNA levels exceed 10,000 per ml on 2 sequential weekly blood draws. Persistently high viremia requires participants to return to their original antiretroviral drug regimen with or without Maraviroc according to recommendations of their HIV care physician.

If participants remain HIV suppressed (below 2,000 vRNA copies per ml of plasma) for >28 days on Maraviroc monotherapy, they will be asked to gradually reduce Maraviroc dosing over a period of 4 weeks followed by intensive monitoring for an additional 28 days. Subjects who maintained HIV suppression with Maraviroc monotherapy are considered to have a functional cure. Subjects who maintain HIV suppression even after Maraviroc withdrawal also have a functional cure. Monthly monitoring for 6 months followed by less intensive monitoring will establish the durability of functional cure.

Sequences

The following sequences are referred to herein:

SEQ ID NO:	Description	Sequence
1	miR30 CCR5	AGGTATATTGCTGTTGACAGTGAGCGACTGTAAAC TGAGCTTGCTCTACTGTGAAGCCACAGATGGGTAG AGCAAGCACAGTTTACCGCTGCCTACTGCCTCGGA CTTCAAGGGGCTT
2	miR21 Vif	CATCTCCATGGCTGTACCACCTTGTGGGGGATGT GTACTTCTGAACCTGTGTGAATCTCATGGAGTTCA GAAGAACACATCCGCACTGACATTTGGTATCTTT CATCTGACCA
3	miR185 Tat	GGGCCTGGCTCGAGCAGGGGGCGAGGGATTCCGCT TCTTCCTGCCATAGCGTGG TCCCCTCCCCCTATGGCAGGCAGAAGCGGCACCTTC CTCCCAATGACCGCGTCTTCGTCG
4	Elongation Factor-1 alpha (EF1-alpha) promoter	CCGGTGCCTAGAGAAGGTGGCGCGGGGTAAACTG GGAAAGTGATGTCGTGTAAGTCCGCTTTTTC CGAGGGTGGGGAGAACCGTATATAAGTGCAGTA GTCGCGCTGAACGTTCTTTTCGCAACGGGTTTGCC GCCAGAACACAGGTAAGTGGCGTGTGGTTCCCG CGGGCCTGGCCTCTTACGGGTTATGGCCCTTGCGT GCCTTGAACTTACTTCCACGCCCTGGCTGCAGTAC GTGATTCTTGATCCCGAGCTTCGGGTGGAAAGTGG GTGGGAGAGTTTCGAGGCCTTCGCTTAAGGAGCCC CTTCGCCCTCGTGCCTTGAGTTGAGGCCCTGGCCTGGG CGCTGGGGCCGCCGCTGCGAATCTGGTGGCACCT TCGCGCCTGTCTCGCTGCTTTCGATAAGTCTCTAGC CATTTAAATTTTGTATGACCTGCTGCGACGCTTTT TTTCTGGCAAGATAGTCTTGTAAATGCGGGCCAAG ATCTGCACACTGGTATTTCGGTTTTTGGGGCCGCGG GCGGCGACGGGGCCCGTGCCTCCAGCGCACATGT TCGGCGAGGCGGGGCTGCGAGCGCGGCCACCGA GAATCGGACGGGGTAGTCTCAAGCTGGCCGCCCT GCTCTGGTGCCTGGCCTCGCGCCGCCGTGTATCGC CCCGCCCTGGGCGGCAAGGCTGGCCCGGTGCGCAC CAGTTGCGTGAGCGGAAAGATGGCCGCTTCCCGGC CCTGCTGCAGGGAGCTCAAAATGGAGGACGCGGC GCTCGGGAGAGCGGGCGGGTGAGTCACCCACACA AAGGAAAAGGGCCTTTCGCTCTCAGCCGTGCTTT CATGTGACTCCACGGAGTACCGGGCGCCGTCCAGG CACCTCGATTAGTTCTCGAGCTTTTGGAGTACGTCG TCTTTAGGTTGGGGGAGGGGTTTTATGCGATGGA GTTTCCCGACACTGAGTGGGTGGAGACTGAAGTTA GGCCAGCTTGGCACTTGATGAATTCCTTGAAT TTGCCCTTTTGTAGTTGGATCTTGGTTCACTCTCA AGCCTCAGACAGTGGTTCAAAGTTTTTCTTCCAT TTCAGGTGTCGTGA
5	CCR5 miRNA target sequence	GAGCAAGCTCAGTTTACA
6	Vif miRNA target sequence	GGGATGTGTACTTCTGAACCTT
7	Tat miRNA target sequence	TCCGCTTCTTCTGCCATAG
8	TAR decoy sequence	CTTGCAATGATGTCGTAATTTGCGTCTTACCTCGTT CTCGACAGCGACCAGATCTGAGCCTGGGAGCTCTC TGGCTGTCAGTAAGCTGGTACAGAAGTTGACGAA AATTCTTACTGAGCAAGAAA
9	Rev/Tat shRNA target sequence	GCGGAGACAGCGACGAAGAGC
10	Rev/Tat shRNA sequence	GCGGAGACAGCGACGAAGAGCTTCAAGAGAGCTC TTCGTCGCTGTCTCCGCTTTTT
11	Gag shRNA target sequence	GAAGAAATGATGACAGCAT
12	Gag shRNA sequence	GAAGAAATGATGACAGCATTTCAAGAGAATGCTGT CATCATTTCTTCTTTTT

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SEQ ID NO:	Description	Sequence
13	Pol shRNA target sequence	CAGGAGCAGATGATACAG
14	Pol shRNA sequence	CAGGAGATGATACAGTTCAAGAGACTGTATCATCT GCTCCTGTTTTT
15	CCR5 shRNA target sequence #1	GTGTCAAGTCCAATCTATG
16	CCR5 shRNA sequence #1	GTGTCAAGTCCAATCTATGTTCAAGAGACATAGAT TGGACTTGACACTTTTT
17	CCR5 shRNA target sequence #2	GAGCATGACTGACATCTAC
18	CCR5 shRNA sequence #2	GAGCATGACTGACATCTACTTCAAGAGAGTAGATG TCAGTCATGCTCTTTTT
19	CCR5 shRNA target sequence #3	GTAGCTCTAACAGGTTGGA
20	CCR5 shRNA sequence #3	GTAGCTCTAACAGGTTGGATTCAAGAGATCCAACC TGTTAGAGCTACTTTTT
21	CCR5 shRNA target sequence #4	GTTTCAGAACTACCTCTTA
22	CCR5 shRNA sequence #4	GTTTCAGAACTACCTCTTATTCAAGAGATAAGAGG TAGTTTCTGAACTTTTT
23	CCR5 shRNA target sequence #5	GAGCAAGCTCAGTTTACACC
24	CCR5 shRNA sequence #5	GAGCAAGCTCAGTTTACACCTTCAAGAGAGGTGTA AACTGAGCTTGCTCTTTTT
25	<i>Homo sapiens</i> CCR5 gene, sequence 1	ATGGATTATCAAGTGTCAAGTCCAATCTATGACAT CAATTATTATACATCGGAGCCCTGCCAAAAAATCA ATGTGAAGCAAATCGCAGCCGCTCTGCTCCG CTCTACTCACTGGTGTTTCATCTTTGGTTTTGTGGGC
26	<i>Homo sapiens</i> CCR5 gene, sequence 2	AACATGCTGGTCATCCTCATCCTGATAAACTGCAA AAGGCTGAAGAGCATGACTGACATCTACCTGCTCA ACCTGGCCATCTCTGACCTGTTTTCTCTTCTACTG TCCCCCTCTGGGCTCACTATGCTGCCGCCAGTGGG ACTTTGGAATACAATGTGTCAACTTTGACAGGG CTCTATTTTATAGGCTTCTCTCTGGAATCTTCTTCA TCATCCTCCTGACAATCGATAGGTACCTGGCTGTC GTCCATGCTGTGTTTGCTTTAAAAGCCAGGACGGT CACCTTTGGGGTGGTGACAAGTGTGATCACTTGGG TGGTGGCTGTGTTTGCCTCTCTCCAGGAATCATCT TTACCAGATCTCAAAAAGAAGGTCTTCATTACACC TGCAGCTCTCATTTTCCATACAGTCAGTATCAATTC TGGAAGAATTTCCAGACATTAAAGATAGTCATCTT GGGGCTGGTCTGCCGCTGCTTGTCTGCTCATCTG CTACTCGGGAATCTTAAAACTCTGCTTCGGTGTG GAAATGAGAAGAAGAGGCACAGGGCTGTGAGGCT TATCTTACCATCATGATTGTTATTTCTCTTCTGG GCTCCCTACAACATTGCTCTCTCTGAAC
27	<i>Homo sapiens</i> CCR5 gene, sequence 3	ACCTTCCAGGAATTCTTTGGCCTGAATAATTGCAGT AGCTCTAACAGGTTGGACCAAGCTATGCAGGTGA
28	<i>Homo sapiens</i> CCR5 gene, sequence 4	CAGAGACTCTTGGGATGACGCACTGCTGCATCAAC CCCATCATCTATGCCTTTGTGCGGGAGAAGTTCAG AAACTACCTTGTAGTCTTCTTCCAAAAGCATTGC CAAACGCTTCTGCAATGCTGTTCTATTTTCCAG

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SEQ ID NO:	Description	Sequence
29	<i>Homo sapiens</i> CCR5 gene, sequence 5	CAAGAGGCTCCCGAGCGAGCAAGCTCAGTTTACAC CCGATCCACTGGGGAGCAGGAAATATCTGTGGGCT TGTGA
30	CD4 promoter sequence	TGTTGGGGTTCAAATTTGAGCCCCAGCTGTTAGCC CTCTGCAAAGAAAAAAAAAAAAAAAAAAGAACAA AGGGCCTAGATTTCCCTTCTGAGCCCCACCCTAAG ATGAAGCCTCTTCTTCAAGGGAGTGGGGTTGGGG TGGAGGCGGATCCTGTCACTTTGCTCTCTCTGTGG CTGGCAGTTTCTCAAAGGGTAACAGGTGTCAAGCT GGCTGAGCCTAGGCTGAACCTGAGACATGTACCT TCTGTCTTCTCATGGCTGGAGGCAGCCTTTGTAAGT CACAGAAAGTAGCTGAGGGGCTCTGGAAGAAAGA CAGCCAGGGTGGAGGTAGATTGGTCTTTGACTCCT GATTTAAGCCTGATTCTGCTTAACCTTTTCCCTTGA CTTTGGCATTTCACCTTTGACATGTTCCCTGAGAGC CTGGGGGGTGGGGAACCCAGCTCCAGCTGGTGACG TTTGGGGCGGCCAGGCCTAGGGTGTGGAGGAGC CTTGCCATCGGGCTTCTGTCTCTTTCATTTAAGC ACGACTCTGCAGA
31	miR30- CCR5/miR21- Vif/miR185 Tat microRNA cluster sequence	AGGTATATGTGCTGTGACAGTGAGCGACTGTAAAC TGAGCTTGCTCTACTGTGAAGCCACAGATGGGTAG AGCAAGCACAGTTTACCCTGCTTACTGCTTCGGA CTTCAAGGGGCTTCCCGGCATCTCCATGGCTGTA CCACCTTGTCTGGGGATGTGTACTTCTGAACCTTGTG TTGAATCTCATGGAGTTCAAGAAGACACATCCGCA CTGACATTTTGGTATCTTTCATCTGACCAGCTAGCG GGCCTGGCTCGAGCAGGGGGCGAGGGATTCCGCTT CTTCTGCCATAGCGTGGTCCCTCCCTATGGCAG GCAGAAGCGGCACCTTCCCTCCCAATGACCGCGTC TTCGTC
32	Long WPRE sequence	AATCAACCTCTGATTACAAAATTTGTGAAGATTG ACTGGTATTCTTAACTATGTTGCTCCTTTTACGCTA TGTGGATACGCTGCTTTAATGCCTTTGTATCATGCT ATTGCTTCCCGTATGGCTTTTCACTTTCTCCTCCTTGT ATAAATCCTGGTTGCTGTCTTTATGAGGAGTTGT GGCCCGTTGTCAAGCAACGTGGCGTGGTGTGCACT GTGTTTGTGACGCAACCCCACTGGTTGGGGCAT TGCCACCACCTGTCACTCCTTTCCGGGACTTTTCGC TTTCCCTCCTTATGCGACGGCGGAACATCATCGC CGCCTGCCTTGCCCGCTGCTGGACAGGGGCTCGGC TGTTGGGCACTGACAATTCGTGGTGTGTGCGGG AAATCATCGTCTTCTTCTTGGCTGCTCGCCTGTGTT GCCACCTGGATTCTGCGCGGACGTCCTTCTGCTA CGTCCCTTCGGCCCTCAATCCAGCGGACCTTCCTTC CCGCGCCTGTGCGGCTCTGCGGCTCTTCCGC GTCTTCGCTTCCGCTCAGACGAGTCGATCTCCC TTTGGGCCCTCCCGCCT
33	Elongation Factor-1 alpha (EF1-alpha) promoter; miR30CCR5; miR21Vif; miR185 Tat	CCGGTGCCTAGAGAAGGTGGCGCGGGGTAACTG GGAAAGTGATGCTGTACTGGCTCCGCCTTTTCC CGAGGGTGGGGGAGAACCGTATATAAGTGCAGTA GTCGCCGTGAACGTTCTTTTTCGCAACGGGTTTGCC GCCAGAACACAGGTAAGTGCCGTGTGTGGTTCCCG CGGGCTGGCCTCTTTACGGGTTATGGCCCTTGCGT GCCTTGAATTACTTCCACGCCCTGGCTGCAGTAC GTGATTCTTGATCCCGAGCTTCGGGTGGAAGTGG GTGGGAGAGTTTCAGGCCTTGCGCTTAAGGAGCCC CTTCGCCCTCGTGTGAGTTGAGGCCTGGCCTGGG CGCTGGGGCGCGCGTGCGAATCTGGTGGCACCT TCGCGCCTGTCTCGCTGCTTTCGATAAGTCTCTAGC CATTTAAATTTTGTATGACCTGCTGCGACGCTTTT TTTCTGGCAAGATAGTCTTGTAAATGCGGGCCAAG ATCTGCACACTGGTATTTTCGGTTTTTGGGGCCGCG GCGGCGACGGGGCCCGTGCCTCCAGCGCACATGT TCGGCGAGGCGGGCCTGCGAGCGCGGCCACCGA GAATCGGACGGGGGTAGTCTCAAGCTGGCCGGCCT GCTCTGGTGCCTGGCCTCGCGCCCGGTGTATCGC CCCGCCTGGGCGGCAAGGCTGGCCCGGTGCGCAC CAGTTGCGTGAGCGGAAGATGGCCGCTTCCCGGC CCTGCTGCAGGAGCTCAAAATGAGGACGCGGC GCTCGGGAGAGCGGGCGGGTGAAGTCAACCAACA AAGGAAAAGGCCCTTCCGTCCTCAGCCGTGCTT

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SEQ ID NO:	Description	Sequence
		CATGTGACTCCACGGAGTACCGGGCGCCGTCCAGG CACCTCGATTAGTTCTCGAGCTTTTGGAGTACGTCG TCTTTAGGTTGGGGGAGGGGTTTTATGCGATGGA GTTTCCCCACACTGAGTGGGTGGAGACTGAAGTTA GGCCAGCTTGGCACTTGATGTAATTCCTTGGAAT TTGCCCTTTTGTAGTTTGGATCTTGGTTCATTCTCA AGCCTCAGACAGTGGTTCAAAGTTTTTCTTCCAT TTCAGGTGTCGTGATGTACAAGGTATATGTCTGTG ACAGTGAGCGACTGTAAGTGTGCTTGTCTACTG TGAAGCCACAGATGGGTAGAGCAAGCACAGTTTAC CGCTGCCTACTGCCTCGGACTTCAAGGGGCTTCCC GGGCATCTCCATGGCTGTACCACCTTGTGCGGGGA TGTGTACTTCTGAACTTGTGTGAATCTCATGGAGT TCAGAAGAACACATCCGCACTGACATTTTGGTATC TTTCATCTGACCAGCTAGCGGGCTTGGCTCGAGCA GGGGCGAGGGATTCCGCTTCTTCTGCCATAGCG TGGTCCCCTCCCCATGGCAGGCAGAAGCGGCACC TCCCTCCCAATGACCGCGTCTTCGTC
34	Rous Sarcoma virus (RSV) promoter	GTAGTCTTATGCAATACTCTTGTAGTCTTGCAACAT GGTAACGATGAGTTAGCAACATGCCCTACAAGGAG AGAAAAAGCACCGTGCATGCCGATTGGTGGAAAT AAGGTGGTACGATCGTGCCTTATTAGGAAGGCAAC AGACGGGTCTGACATGGATTGGACGAACCACTGAA TTGCCGATTGCAGAGATATTGTATTAAAGTGCCTA GCTCGATACAATAAACG
35	5' Long terminal repeat (LTR)	GGTCTCTCTGGTTAGACCAGATCTGAGCCTGGGAG CTCTCTGGCTAACTAGGGAACCCACTGCTTAAGCC TCAATAAAGCTTGCCCTTGTGCTTCAAGTAGTGT GTGCCCCGTCTGTTGTGTGACTCTGGTAACTAGAGA TCCCTCAGACCCTTTTAGTCAGTGTGAAAAATCTCT AGCA
36	Psi Packaging signal	TACGCCAAAAATTTTACTAGCGGAGGCTAGAAGG AGAGAG
37	Rev response element (RRE)	AGGAGCTTTGTTCCTTGGGTTCTTGGGAGCAGCAG GAAGCACTATGGGCGCAGCCTCAATGACGCTGACG GTACAGGCCAGACAATTATTGTCTGTATAGTGCA GCAGCAGAACAATTGTCTGAGGGCTATTGAGGCGC AACAGCATCTGTTGCAACTCACAGTCTGGGGCATC AAGCAGCTCCAGGCAAGAATCTGGCTGTGGAAA GATACCTAAAGGATCAACAGCTCC
38	Central polypurine tract (cPPT)	TTTTAAAAGAAAAGGGGGATTGGGGGTACAGT GCAGGGGAAAGAAATAGTAGACATAATAGCAACAG ACATACAAACTAAAGAATTACAAAAACAAATTAC AAAATTCAAATTTTA
39	3' delta LTR	TGGAAGGGCTAATTCACCTCCACGAAGATAAGAT CTGCTTTTGTCTGTACTGGGTCTCTGTTAGAC CAGATCTGAGCCTGGGAGCTCTCTGGCTAACTAGG GAACCCACTGCTTAAGCCTCAATAAAGCTTGCCTT GAGTGTCTCAAGTAGTGTGTGCCGCTGTGTGTGTG ACTCTGGTAAC TAGAGATCCCTCAGACCCTTTTAGT CAGTGTGAAAATCTCTAGCAGTAGTAGTTATGT CA
40	Helper/Rev; CMV early (CAG) enhancer; Enhance Transcription	TAGTTATTAATAGTAATCAATTACGGGGTCATTAG TTCATAGCCCATATATGGAGTTCCGCGTTACATAA CTTACGGTAAATGGCCCGCCTGGCTGACCGCCAA CGACCCCGCCCAATTGACGTCAATAATGACGTATG TTCCCATAGTAACGCCAATAGGGACTTCCATTGA CGTCAATGGGTGGACTATTACGGTAAACTGCCCA CTTGGCAGTACATCAAGTGATCATATGCCAAGTA CGCCCCCTATTGACGTCAATGACGGTAAATGGCCC GCCTGGCATTATGCCAGTACATGACCTTATGGGA CTTTCCTACTTGGCAGTACATCTACGTATTAGTCAT C
41	Helper/Rev; Chicken beta actin (CAG)	GCTATTACCATGGGTGAGGTGAGCCCCACGTTCT GCTTCACTCTCCCATCTCCCCCCTCCCCACCCC CAATTTTGTATTTATTTATTTTAAATTATTTTGTGC

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SEQ ID NO:	Description	Sequence
	promoter; Transcription	AGCGATGGGGGCGGGGGGGGGGGGGCGCGCGCC AGGCGGGGCGGGGCGGGGCGAGGGGCGGGGCGGG GCGAGGCGGAGAGGTGCGGCGGCAGCCAATCAGA GCGGCGCGCTCCGAAAGTTTCTTTTATGGCGAGG CGGCGGCGGCGGCGGCCCTATAAAAAGCGAAGCG CGCGGCGGGCG
42	Helper/Rev; Chicken beta actin intron; Enhance gene expression	GGAGTCGCTGCGTTGCTTTCGCCCCGTGCCCGCTC CGCGCCGCTCGCGCCGCGCCCGCGGCTCTGACT GACCGGTACTCCACAGGTGAGCGGGCGGGACG GCCCTTCTCTCCGGGCTGTAATTAGCGCTTGGTTT AATGACGGCTCGTTTCTTTCTGTGGCTGCGTGA GCCTTAAAGGGCTCCGGGAGGGCCCTTGTGCGGG GGGGAGCGGCTCGGGGGTGCCTGCGTGTGTGTGT GCGTGGGGAGCGCGCGTGCAGGCGCGCTGCCCG GCGGCTGTAGCGCTGCGGCGCGCGCGGGGCTT TGTGCGCTCCGCGTGTGCGCGAGGGGAGCGCGGCC GGGGGCGGTGCCCCGCGGTGCGGGGGGCTGCGA GGGGAACAAGGCTGCGTGCAGGGTGTGTGCGTG GGGGGTGAGCAGGGGTGTGGGCGCGGCGGTGCG GGCTGTAAACCCCCCTGCACCCCCCTCCCGAGT TGCTGAGCACGGCCCGGCTTCGGGTGCGGGCTCC GTGCGGGCGTGGCGCGGGGCTCGCCGTGCGGGC GGGGGTGGCGGCGAGTGGGGTGCAGGGCGGGG CGGGCGCGCTCGGGCGGGGAGGCTCGGGGA GGGGCGCGGCGGCGCGGAGCGCGGCGGCTGTC GAGGCGCGGCGAGCCGAGCATTGCCCTTTATGG TAATCGTGCAGAGGGCGCAGGGACTTCTTTGTG CCAAATCTGGCGAGCCGAAATCTGGGAGGCGCC GCCGACCCCCCTTAGCGGGCGCGGGCGAAGCGGT GCGGCGCGGCGAGGAAGGAAATGGCGGGGAGGG CCTTCGTGCGTGCAGCGCGCGCCCTTCTCCA TCTCCAGCTCGGGGCTGCGCGAGGGGACGGCTG CCTTCGGGGGGACGGGGCAGGGCGGGTTGCGC TTCTGCGTGTGACCGGCGG
43	Helper/Rev; HIV Gag; Viral capsid	ATGGGTGCGAGAGCGTCAGTATTAAGCGGGGAG AATTAGATCGATGGGAAAAAATTCGGTTAAGGCCA GGGGGAAGAAAAAATATAAATTAAACATATAG TATGGCAAGCAGGGAGCTAGAAGCATTCGAGTT AATCCTGGCTGTAGAAACATCAGAAGGCTGTAG ACAAATACTGGGACAGCTACAACCATCCCTTCAGA CAGGATCAGAAGAACTTAGATCATTATATAATACA GTAGCAACCCCTCTATTGTGTCATCAAAGGATAGA GATAAAGACACCAAGGAAGCTTTAGACAAGATA GAGGAAGACCAAAACAAAAGTAAGAAAAAGCAC AGCAAGCAGCAGCTGACACAGGACACAGCAATCA GGTCAGCCAAATTAACCTATAGTGCAGAACATCC AGGGCAATGGTACATCAGGCCATATCACCTAGA ACTTTAATGCATGGGTAAAAGTAGTAGAAGAGA AGGCTTTCAGCCAGAGTGATACCCATGTTTTC GCATTATCAGAAGGAGCCACCCACAAGATTAAA CACCATGCTAAACACAGTGGGGGACATCAAGCA GCCATGCAAAATGTTAAAGAGACCATCAATGAGG AAGCTGCAGAAATGGGATAGAGTGCATCCAGTGCAT GCAGGGCTATTGCACCGGCGAGTGAAGAAC CAAGGGGAAGTGACATAGCAGGAACCTAGTAGTAC CCTTCAGGAACAAATAGGATGGATGACACATAATC CACCTATCCAGTAGGAGAAATCTATAAAGATGG ATAATCTGGGATTAATAAATAGTAAGAATGTA TAGCCCTACCAGCATCTGGACATAAGACAAGGAC CAAAGGAACCCCTTTAGAGACTATGTAGACCGATT TATAAACTCTAAGAGCCGAGCAAGCTTCAAGA GGTAAAAAATTGGATGACAGAACTTGTGGTCC AAAATGCGAACCCAGATTGTAAGACTATTTAAAA GCATTGGGACCAGGAGCGACACTAGAAGAAATGA TGACAGCATGTGAGGAGTGGGGGACCCGCGCA TAAAGCAAGAGTTTTGGCTGAAGCAATGAGCCAAG TAACAAATCCAGCTACCATAATGATACAGAAAGGC AATTTAGGAACCAAGAAAGACTGTAAAGTGTTC CAATTGTGGCAAGGAAGGGCACATAGCCAAAAAT TGCAGGGCCCCTAGGAAAAAGGGCTGTTGGAAT GTGAAAGGAAGGACACCAATGAAAGATTGTAC TGAGAGACAGGCTAATTTTATGGGAAGATCTGGC CTTCCACAGGGAAGGCCAGGGAATTTCTTCAG AGCAGACCAGAGCCACAGCCCCACAGAAAGAGA

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SEQ ID NO:	Description	Sequence
		GCTTCAGGTTTGGGGAAGAGACAACAACCTCCCTCT CAGAAGCAGGAGCCGATAGACAAGGAACGTATC CTTTAGCTTCCCTCAGATCACTCTTTGGCAGCGACC CCTCGTCACAATAA
44	Helper/Rev; HIV Pol; Protease and reverse transcriptase	ATGAATTGCCCAGGAAGATGGAAACCAAAATGA TAGGGGGAATTGGAGGTTTATCAAAGTAGGACAG TATGATCAGATACTCATAGAAATCTGCGACATAA AGCTATAGGTACAGTATTAGTAGGACCTACACCTG TCAACATAATTGGAAGAAATCTGTTGACTCAGATT GGCTGCACTTTAAATTTTCCCATAGTCCTATTGAG ACTGTACCAGTAAATTAAGCCAGGAATGGATGG CCCAAAAGTTAAACAATGGCCATTGACAGAAGAA AAAAATAAGCATTAGTAGAAATTTGTACAGAAAT GGAAAAGGAAGGAAAAATTTCAAAATTTGGGCCT GAAATCCATACAACTACTCCAGTATTGCCCATAA GAAAAAGACAGTACTAAATGGAGAAAATTAGTA GATTTAGAGAACTTAATAAGAGAACTCAAGATTT CTGGGAAGTTCAATTAGGAATACCACATCCTGCAG GGTTAAAAACAGAAAAATCAGTAACAGTACTGGA TGTTGGGCGATGCATATTTTTCAGTTCCCTTAGATAA AGACTTCAGGAAGTATACTGCATTACCATACCTA GTATAACAATGAGACACCGGGATTAGATATCAG TACAATGTGCTCCACAGGGATGGAAAGGATCACC AGCAATATTCCAGTGTAGCATGACAAAAATCTTAG AGCCTTTTAGAAAAACAAATCCAGACATAGTCATC TATCAATACATGGATGATTTGTATGTAGGATCTGA CTTAGAAATAGGGCAGCATAGAACAAAAATAGAG GAACTGAGACAACATCTGTTGAGGTGGGATTTAC CACACCAGACAAAAACATCAGAAAGAACCTCCA TTCCTTTGGATGGGTTATGAACTCCATCCTGATAAA TGGACAGTACAGCCTATAGTGCTGCCAGAAAAGGA CAGCTGGACTGTCAATGACATACAGAAATTAGTGG GAAATTTGAATTGGGCAAGTCAGATTTATGCAGGG ATTAAAGTAAGGCAATTATGTAACCTTCTTAGGGG AACCAAAGCACTAACAGAAGTAGTACCCTAACCA GAAGAAGCAGAGCTAGAACTGGCAGAAAACAGGG AGATTCTAAAAGAACCGGTACATGGAGTGTATTAT GACCCATCAAAAGACTTAATAGCAGAAATACAGA AGCAGGGGCAAGGCCAATGGACATATCAAAATTTAT CAAGAGCCATTAAAAATCTGAAAACAGGAAAAT ATGCAAGAATGAAGGGTGCCCACTAATGATGTG AAACAATTACAGAGGCAGTACAAAAATAGCCA CAGAAAGCATAGTAATATGGGGAAGACTCCTAA ATTTAAATTACCCATACAAAAGGAAACATGGGAAG CATGGTGGACAGAGTATTGGCAAGCCACCTGGATT CCTGAGTGGGAGTTTGTCAATACCCCTCCCTTAGTG AAGTTATGGTACCAGTTAGAGAAAGAACCCATAAT AGGAGCAGAACTTTCTATGTAGATGGGGCAGCCA ATAGGGAAACTAAATTAGGAAAAGCAGGATATGT AACTGACAGAGGAAGACAAAAGTTGTCCCTTA ACGGACACACAAATCAGAAGACTGAGTTACAAG CAATTCATCTAGCTTTGCAGGATTCGGGATTAGAA GTAAACATAGTGACAGACTCACAATATGCATTGGG AATCATTCAAGCACAAACCAGATAAGAGTGAATCAG AGTTAGTCAGTCAAAATAATAGAGCAGTTAATAAAA AAGGAAAAGTCTACCTGGCATGGGTACCAGCAC ACAAAGGAATTGGAGGAAATGAACAAGTAGATGG GTTGGTCAGTGCTGGAATCAGGAAAGTACTA
45	Helper Rev; HIV Integrase; Integration of viral RNA	TTTTTAGATGGAATAGATAAGGCCCAAGAAGACA TGAGAAATATCACAGTAATTGGAGAGCAATGGCTA GTGATTTTAACCTACCCTGTAGTAGCAAAAGAA ATAGTAGCCAGCTGTATAAATGTCAGCTAAAAGG GGAAGCCATGCATGGACAAGTAGACTGTAGCCCA GGAATATGGCAGCTAGATTGTACACATTTAGAAGG AAAAGTTATCTTGGTAGCAGTTTCATGTAGCCAGTG GATATATAGAAGCAGAAGTAATTCCAGCAGAGAC AGGGCAAGAAACAGCATACTTCCTCTTAAATTAG CAGGAAGATGGCCAGTAAAAACAGTACATACAGA CAATGGCAGCAATTTACCAGTACTACAGTTAAGG CCGCCTGTGGTGGGCGGGATCAAGCAGGAATTT GGCATTCCCTACAATCCCAAGTCAAGGAGTAAT AGAATCTATGAATAAAGAAATTAAGAAAATATAG GACAGGTAAGAGATCAGGCTGAACATCTTAAGAC AGCAGTACAAATGGCAGTATTCATCCACAATTTTA

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SEQ ID NO:	Description	Sequence
		AAAGAAAAGGGGGATTGGGGGTACAGTGCAGG GGAAAGAATAGTAGACATAATAGCAACAGACATA CAAACAAAGAATTACAAAAACAAATTACAAAAA TTCAAAATTTTCGGGTTTATTACAGGGACAGCAGA GATCCAGTTTGGAAAGGACCAGCAAAGCTCCTCTG GAAAGGTGAAGGGGCAGTAGTAATACAGATAAT AGTGACATAAAGTAGTGCCAGAAGAAAAGCAA AGATCATCAGGGATTATGGAACAGATGGCAGG TGATGATTGTGTGGCAAGTAGACAGGATGAGGATT AA
46	Helper/Rev; HIV RRE; Binds Rev element	AGGAGCTTTGTTCCTTGGGTTCTTGGGAGCAGCAG GAAGCACTATGGGCGCAGCGTCAATGACGCTGACG GTACAGGCCAGACAATTATTGTCTGGTATAGTGCA GCAGCAGAACAAATTGCTGAGGGCTATTGAGGCGC AACAGCATCTGTTGCAACTCACAGTCTGGGGCATC AAGCAGCTCCAGGCAAGAATCCTGGCTGTGGAAA GATACCTAAAGGATCAACAGCTCCT
47	Helper/Rev; HIV Rev; Nuclear export and stabilize viral mRNA	ATGGCAGGAAGAAGCGGAGACAGCGACGAAGAAC TCCTCAAGGCAGTCAGACTCATCAAGTTTCTCTATC AAAGCAACCCACCTCCCAATCCCGAGGGGACCCG ACAGGCCCGAAGGAATAGAAGAAGAAGTGGAGA GAGAGACAGAGACAGATCCATTTCGATTAGTGAAC GGATCCTTAGCACTTATCTGGGACGATCTGCGGAG CCTGTGCCTCTTCAGCTACCACCGCTTGAGAGACTT ACTCTTGATTGTAACGAGGATTGTGGAACCTCTGG GACGCAAGGGGTGGGAAGCCCTCAAATATTGGTG GAATCTCTACAATATTGGAGTCAGGAGCTAAAGA ATAG
48	Helper/Rev; Rabbit beta globin poly A; RNA stability	AGATCTTTTCCCTCTGCCAAAAATTATGGGGACAT CATGAAGCCCCCTTGAGCATCTGACTTCTGGCTAAT AAAGGAAATTTATTTTCATTGCAATAGTGTGTGG AATTTTTTGTGTCTCTCACTCGGAAGGACATATGGG AGGGCAATCATTTAAACATCAGAATGAGTATTT GGTTTAGAGTTTGGCAACATATGCCATATGCTGGC TGCCATGAACAAAGGTGGCTATAAAGAGGTCACTA GTATATGAACAGCCCCCTGCTGTCCATTCTTTATT CCATAGAAAAGCCTTGACTTGAGGTTAGATTTTTTT TATATTTTGTTTTGTGTATTTTTTTCTTTAACATCC CTAAAATTTTCTTACATGTTTTACTAGCCAGATTT TTCTCTCTCTCTGACTACTCCAGTCATAGCTGTC CCTCTTCTCTTATGAAGATC
49	Helper; CMV early (CAG) enhancer; Enhance transcription	TAGTTATTAATAGTAATCAATTACGGGGTCATTAG TTCATAGCCCATATATGGAGTTCGCGTTACATAA CTTACGGTAAATGGCCCGCCTGGCTGACCGCCCAA CGACCCCGCCCATTGACGTCAATAATGACGTATG TTCCCATAGTAACGCCAATAGGGACTTTCCATTGA CGTCAATGGGTGGACTATTTACGGTAAACTGCCCA CTTGGCAGTACATCAAGTGATCATATGCCAAGTA CGCCCCCTATTGACGTCAATGACGGTAAATGGCCC GCCTGGCATTATGCCAGTACATGACCTTATGGGA CTTCTCTACTTGGCAGTACATCTACGTATTAGTCAT C
50	Helper; Chicken beta actin (CAG) promoter; Transcription	GCTATTACCATGGGTGAGGTGAGCCCCACGTTCT GCTTCACTCTCCCCATCTCCCCCCCCCTCCACCCCC CAATTTTGATTTTATTTATTTTAAATATTTTGTGC AGCGATGGGGCGGGGGGGGGGGGGCGCGCGCC AGGCGGGGCGGGGCGGGGCGAGGGGCGGGGCGGG GCGAGGCGGAGAGGTGCGGCGGCAGCCAATCAGA GCGGCGCGCTCCGAAAGTTTCCTTTTATGGCGAGG CGGCGGCGGCGGCGGCCCTATAAAAAGCGAAGCG CGCGCGGGCG
51	Helper; Chicken beta actin intron; Enhance gene expression	GGAGTCGCTGCGTTGCCTTCGCCCCGTGCCCCGCTC CGCGCCGCTCGCGCCCGCCCGCCCGGCTCTGACT GACCGGTTACTCCACAGGTGAGCGGGCGGGACG GCCCTTCTCTCCGGCTGTAATTAGCGCTTGGTTT AATGACGGCTCGTTCTTTTCTGTGGCTGCGTGAAA GCCTTAAAGGCTCCGGGAGGGCCCTTGTGCGGG GGGGAGCGGCTCGGGGGGTGCGTGCCTGTGTGT GCGTGGGAGCGCGCGTGCAGCCCGCGCTGCCCG GCGGCTGTGAGCGCTGCGGGCGCGCGCGGGGCTT

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SEQ ID NO:	Description	Sequence
		TGTGCGCTCCGCGTGTGCGCGAGGGGAGCGCGGCC GGGGGCGGTGCCCCGCGGTGCGGGGGGGCTGCGA GGGGAACAAAGGCTGCGTGCAGGGGTGTGTGCGTG GGGGGGTGAGCAGGGGGTGTGGGCGCGCGGTCTG GGCTGTAACCCCCCTGCACCCCCCTCCCGAGT TGCTGAGCACGGCCCGCTTCGGGTGCGGGGCTCC GTGCGGGCGTGGCGGGGGTCTGCCGTGCCGGGC GGGGGGTGGCGGCAGGTGGGGTGCCGGGCGGGG CGGGCCCGCTCGGGCCGGGAGGGCTCGGGGA GGGGCGCGCGGCCCGGAGCGCCGGCGGTGTCTC GAGGCGCGGCGAGCCGAGCCATTGCCTTTTATGG TAATCGTGCGAGAGGCGCAGGGACTTCCTTTGTC CCAAATCTGGCGAGCCGAAATCTGGGAGGCGCC GCCGCACCCCTCTAGCGGGCGCGGGCGAAGCGGT GCGGCGCGGCGAGGAAGAAATGGGCGGGGAGGG CCTTCGTGCGTGCCTGCGCGCGCTCCCTTCTCCA TCTCCAGCCTCGGGGCTGCCGAGGGGACGGCTG CCTTCGGGGGGACGGGCGAGGGCGGGTTTCGGC TTCTGGCGTGTGACCGGCGG
52	Helper; HIV Gag; Viral capsid	ATGGGTGCGAGAGCGTCAGTATTAAGCGGGGAG AATTAGATCGATGGGAAAAAATTCGGTTAAGGCCA GGGGGAAGAAAAATATAAATTAACATATAG TATGGGCAAGCAGGGAGCTAGAACGATTTCGAGTT AATCCTGGCCTGTTAGAAACATCAGAAGGCTGTAG ACAAACTACTGGGACAGCTACAACCATCCCTTCAGA CAGGATCAGAAAGACTTAGATCATTATATAATACA GTAGCAACCTCTATTGTGTGCATCAAGGATAGA GATAAAGACACCAAGGAAGCTTTAGACAAGATA GAGGAAGAGCAAAACAAAGTAAGAAAAAGCAC AGCAAGCAGCAGCTGACACAGGACACAGCAATCA GGTGAGCCAAAATTACCTATAGTGCAGAACATCC AGGGGCAATGGTACATCAGGCCATATCACCTAGA ACTTTAATGCATGGGTAAAAGTAGTAGAAGAGA AGGCTTTCAGCCAGAGTGATACCCATGTTTTCA GCATTATCAGAAGAGCCACCCACAAGATTTAAA CACCATGCTAAACACAGTGGGGGACATCAAGCA GCCATGCAAAATGTTAAAAGAGACCATCAATGAGG AAGCTGCAGAAATGGGATAGAGTGCATCCAGTGCAT GCAGGGCCTATTGCACCAGGCCAGATGAGAGAAC CAAGGGGAAGTGACATAGCAGGAACACTAGTAC CCTTCAGGAACAAATAGGATGGATGACACATAATC CACCTATCCCAGTAGGAGAAATCTATAAAGATGG ATAATCCTGGGATTAAATAAAATAGTAAGAATGTA TAGCCCTACCGACTTCTGGACATAAGACAAGGAC CAAAGGAACCTTTAGAGACTATGTAGACCGATTCT TATAAACTCTAAGAGCCGAGCAAGCTTCACAAGA GGTAAAAAATGGATGACAGAAACCTTGTTGGTCC AAAATGCGAACCCAGATTGTAAGACTATTTAAAA GCATTGGGACCAGGAGCGCACTAGAAGAAATGA TGACAGCATGTGAGGAGTGGGGGACCCGGCCA TAAAGCAAGAGTTTGGCTGAAGCAATGAGCCAAG TAACAAATCCAGCTACCATAATGATACAGAAAGGC AATTTAGGAACCAAGAAAGAGCTGTTAAGTGTTC CAATTGTGGCAAGGAAGGGCACATAGCCAAAAAT TGCAGGGCCCCTAGGAAAAAGGCTGTTGGAAT GTGGAAAGGAAGGACACAAATGAAAGATTGTAC TGAGAGACAGGCTAATTTTTAGGGAAGATCTGGC CTTCCCAAGGGAAGGCCAGGGAATTTCTTCAG AGCAGACCAGAGCCAACAGCCCCACAGAAGAGA GCTTCAGGTTTGGGAAGAGACAACAACCTCCTCT CAGAAGCAGGAGCCGATAGACAAGGAACGTATC CTTTAGCTTCCCTCAGATCACTCTTTGGCAGCGACC CCTCGTCACAATAA
53	Helper; HIV Pol; Protease and reverse transcriptase	ATGAATTTGCCAGGAAGATGGAAACCAAAATGA TAGGGGGAATGGAGGTTTTATCAAAGTAGGACAG TATGATCAGATACTCATAGAAATCTCGGCACATAA AGCTATAGGTACAGTATTAGTAGGACCTACACCTG TCAACATAATTGGAAGAAATCTGTTGACTCAGATT GGCTGCCTTTAAATTTTCCATTAGTCCTATTGAG ACTGTACCAGTAAAAATTAAAGCCAGGAATGGATGG CCCAAAGTTAAACAATGGCCATTGACAGAAGAA AAAATAAAGCATTAGTAGAAATTTGTACAGAAAT GGAAGGAAGGAAGAAATTTCAAAATTTGGCCT GAAATCCATACAATACTCCAGTATTTGCCATAAA

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SEQ ID NO:	Description	Sequence
		GAAAAAGACAGTACTAAATGGAGAAAATTAGTA GATTCAGAGAACTTAATAAGAGAACTCAAGATTT CTGGGAAGTTCAATTAGGAATACCACATCCTGCAG GGTTAAACAGAAAAATCAGTAACAGTACTTGA TGTGGGCGATGCATATTTTCAGTTCCTTAGATAA AGACTTCAGGAAGTATACTGCATTACCATACCTA GTATAACAATGAGACACCGGATTAGATATCAG TACAATGTGCTTCCACAGGGATGGAAAGGATCACC AGCAATATTCAGTGTAGCATGACAAAAATCTTAG AGCCTTTTAGAAAAACAAATCCAGACATAGTCATC TATCAATACATGGATGATTTGTATGTAGGATCTGA CTTAGAAATAGGGCAGCATAGAACAAAAATAGAG GAACTGAGACAACATCTGTTGAGGTGGGATTAC CACACCAGACAAAAACATCAGAAAGAACTCCA TTCCTTTGGATGGGTATGAACTCCATCCTGATAAA TGGACAGTACAGCCTATAGTGTGCCAGAAAAGGA CAGCTGGACTGTCAATGACATACAGAAATTAGTGG GAAAAATGAATTGGGCAAGTCAGATTTATGCAGGG ATTAAGTAAGGCAATTATGTAACTTCTTAGGGG AACCAAAGCACTAACAGAAGTAGTACCCTAACA GAAGAAGCAGAGCTAGAACTGGCAGAAAACAGGG AGATTCTAAAGAACCAGTACATGGAGTGTATTAT GACCCATCAAAGACTTAATAGCAGAAATACAGA AGCAGGGGCAAGGCCAATGGACATATCAAAATTTAT CAAGAGCCATTAAAAATCTGAAAACAGGAAAAAT ATGCAAGAATGAAGGTGCCCACTAATGATGTG AAACAATTAAACAGAGGCAGTACAAAAATAGCCA CAGAAAGCATAGTAATATGGGAAAGACTCCTAA ATTTAAATTACCCATACAAAAGGAAACATGGGAAG CATGGTGGACAGATATGGCAAGCCACCTGGATT CCTGAGTGGGAGTTTGTCAATACCCCTCCCTAGTG AAGTTATGGTACCAGTTAGAGAAAGAACCCATAAT AGGAGCAGAACTTTCTATGTAGATGGGGCAGCCA ATAGGGAACATAATTAGGAAAAGCAGGATATGT AACTGACAGAGGAAGACAAAAGTTGTCCCTTA ACGGACACAACAAATCAGAAGACTGAGTTACAAG CAATTCATCTAGCTTTCAGGATTTCGGATTAGAA GTAAACATAGTGACAGACTCACAATATGCATTGGG AATCATTCAGCACAACAGATAAGAGTGAATCAG AGTTAGTCAGTCAAATAATAGAGCAGTTAATAAAA AAGGAAAAAGTCTACCTGGCATGGGTACCAGCAC ACAAGGAATTGGAGGAAATGAACAAGTAGATGG GTTGGTCAGTGCTGGAATCAGGAAAGTACTA
54	Helper; HIV Integrase; Integration of viral RNA	TTTTATAGTGAATAGATAAGGCCCAAGAAGAACA TGAGAAATATCACAGTAATTGGAGAGCAATGGCTA GTGATTTTAACCTACCACCTGTAGTAGCAAAAGAA ATAGTAGCCAGCTGTGATAAATGTCAGCTAAAAGG GGAAGCCATGCATGGACAAGTAGACTGTAGCCCA GGAATATGGCAGCTAGATTGTACACATTTAGAAGG AAAAGTTATCTTGGTAGCAGTTTCATGTAGCCAGTG GATATATAGAAGCAGAAGTAATTCAGCAGAGAC AGGGCAAGAAACAGCATACTTCTCTTAAATTAG CAGGAAGATGGCCAGTAAAAACAGTACATACAGA CAATGGCAGCAATTTACCAGTACTACAGTTAAGG CCGCCTGTGTGGGCGGGATCAAGCAGGAATTT GGCATTCCCTACAATCCCAAAGTCAAGGAGTAAT AGAATCTATGAATAAAGAATTAAAGAAAATTATAG GACAGGTAAGAGATCAGGCTGAACATCTTAAGAC AGCAGTACAAATGGCAGTATTCATCCCAATTTTA AAAGAAAAGGGGGATTGGGGGTACAGTGCAGG GGAAAGAATAGTAGACATAATAGCAACAGACATA CAACTAAAGAATTACAAAAACAAATTACAAAAA TTCAAAATTTTCGGTTTATTACAGGGACAGCAGA GATCCAGTTTGGAAAGGACCAGCAAGCTCCTCTG GAAAGGTGAAGGGCAGTAGTAATACAAGATAAT AGTGACATAAAAGTAGTGCCAAGAAGAAAAGCAA AGATCATCAGGGATTATGGAAAACAGATGGCAGG TGATGATTGTGTGGCAAGTAGACAGGATGAGGATT AA
55	Helper; HIV RRE; Binds Rev element	AGGAGCTTTGTTTCCTTGGGTTCTTGGGAGCAGCAG GAAGCACTATGGGCGCAGCGTCAATGACGCTGACG GTACAGGCCAGACAATTATGTCTGGTATAGTGCA GCAGCAGACAATTGCTGAGGGCTATTGAGGCGC AACAGCATCTGTTGCAACTCACAGTCTGGGGCATC

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SEQ ID NO:	Description	Sequence
		AAGCAGCTCCAGGCAAGAATCCTGGCTGTGGAAA GATACCTAAAGGATCAACAGCTCCT
56	Helper; Rabbit beta globin poly A; RNA stability	AGATCTTTTCCCTCTGCCAAAAATTATGGGGACAT CATGAAGCCCTTGAGCATCTGACTTCTGGCTAAT AAAGGAAATTTATTTTCATTGCAATAGTGTGG AATTTTGTGTCTCTCACTCGGAAGGACATATGGG AGGGCAAATCATTAAAAACATCAGAATGAGTATTT GGTTTAGAGTTTGGCAACATATGCCATATGCTGGC TGCCATGAACAAAGGTGGCTATAAAGAGGTCATCA GTATATGAAACAGCCCCCTGCTGTCCATTCTTTATT CCATAGAAAAGCCTTGACTTGAGGTTAGATTTTTTT TATATTTTGTTTTGTGTTATTTTTTCTTTAACATCC CTAAAATTTTCTTACATGTTTTACTAGCCAGATTT TTCCTCCTCTCCTGACTACTCCAGTCATAGCTGTC CCTCTTCTCTTATGAAGATC
57	Rev; RSV promoter; Transcription	ATGGCAGGAAGAAGCGGAGACAGCGACGAAGAAC TCCTCAAGGCAGTCAGACTCATCAAGTTTCTCTATC AAAGCAACCCACCTCCCAATCCCGAGGGGACCCG ACAGGCCCGAAGGAATAGAAGAAGAAGGTGGAGA GAGAGACAGAGACAGATCCATTTCGATTAGTGAAC GGATCCTTAGCACTTATCTGGGACGATCTGCGGAG CCTGTGCCTCTTCAGCTACCACCGCTTGAGAGACTT ACTCTTGATTGTAACGAGGATTGTGGAACCTCTGG GACGCAGGGGGTGGGAAGCCCTCAAATATTGGTG GAATCTCCTACAATATTGGAGTCAGGAGCTAAAGA ATAG
58	Rev; HIV Rev; Nuclear export and stabilize viral mRNA	ATGGCAGGAAGAAGCGGAGACAGCGACGAAGAAC TCCTCAAGGCAGTCAGACTCATCAAGTTTCTCTATC AAAGCAACCCACCTCCCAATCCCGAGGGGACCCG ACAGGCCCGAAGGAATAGAAGAAGAAGGTGGAGA GAGAGACAGAGACAGATCCATTTCGATTAGTGAAC GGATCCTTAGCACTTATCTGGGACGATCTGCGGAG CCTGTGCCTCTTCAGCTACCACCGCTTGAGAGACTT ACTCTTGATTGTAACGAGGATTGTGGAACCTCTGG GACGCAGGGGGTGGGAAGCCCTCAAATATTGGTG GAATCTCCTACAATATTGGAGTCAGGAGCTAAAGA ATAG
59	Rev; Rabbit beta globin poly A; RNA stability	AGATCTTTTCCCTCTGCCAAAAATTATGGGGACAT CATGAAGCCCTTGAGCATCTGACTTCTGGCTAAT AAAGGAAATTTATTTTCATTGCAATAGTGTGG AATTTTGTGTCTCTCACTCGGAAGGACATATGGG AGGGCAAATCATTAAAAACATCAGAATGAGTATTT GGTTTAGAGTTTGGCAACATATGCCATATGCTGG CTGCCATGAACAAAGGTGGCTATAAAGAGGTCAT CAGTATATGAAACAGCCCCCTGCTGTCCATTCTTTA TTCCATAGAAAAGCCTTGACTTGAGGTTAGATTTTT TTTATATTTTGTTTTGTGTTATTTTTTCTTTAACAT CCCTAAAATTTTCTTACATGTTTTACTAGCCAGAT TTTTCTCCTCTCCTGACTACTCCAGTCATAGCTG TCCCTCTTCTTATGGAGATC
60	Envelope; CMV promoter; Transcription	ACATTGATTATTGACTAGTTATTAATAGTAATCAAT TACGGGGTCATTAGTTTCATAGCCCATATATGGAGT TCCGCGTTACATAACTTACGGTAATAGGCCCGCCT GGCTGACCGCCCAACGACCCCGCCCATGACGTC AATAATGACGTATGTTCCCATAGTAACGCCAATAG GGACTTTCCATTGACGTCAATGGGTGGAGTATTTA CGGTAAACTGCCCCTTGGCAGTACATCAAGTGTA TCATATGCCAAGTACGCCCCCTATTGACGTCAATG ACGGTAAATGGCCCGCCTGGCATTATGCCAGTAC ATGACCTTATGGGACTTTCCTACTTGGCAGTACATC TACGTATTAGTCATCGCTATTACCATGGTGATGCG GTTTTGGCAGTACATCAATGGGCGTGATAGCGGT TTGACTCACGGGGATTTCCAAGTCTCCACCCCATTG ACGTCAATGGGAGTTGTTTTGGCACCAAAATCAA CGGGACTTTCAAAATGTCGTAACAACCTCCGCCCC ATTGACGCAATGGGCGGTAGGCGGTACCGTGGG AGGTCTATATAAGC

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SEQ ID NO:	Description	Sequence
61	Envelope; Beta globin intron; Enhance gene expression	GTGAGTTTGGGGACCCCTGATTGTTCTTTCTTTTTC GCTATTGTAAATTCATGTTATATGGAGGGGGCAA AGTTTTCAGGGTGTGTTTAGAATGGGAAGATGTC CCTTGATCACCATGGACCCCTCATGATAATTTTGTT TCTTTCACCTTCTACTCTGTTGACAACCATTTGCTC CTCTTATTTTCTTTTCATTTTCTGTAACCTTTTCGTT AAACCTTTAGCTTGCAATTTGTAACGAATTTTAAAT CACTTTTGTATTATTGTGAGATTGTAAGTACTTTCT CTAATCACTTTTTTTTCAAGGCAATCAGGGTATATT ATATTGTACTTCAGCACAGTTTGTAGAGAACAATTG TTATAATTAAGTATAAGGTAGAATATTCTGCAT ATAAATTCGGCTGGCGTGGAATATTCTTATTGGT AGAAACAACCTACACCCCTGGTCATCATCTGCCTTT CTCTTTATGGTTACAATGATATACACTGTTTGAGAT GAGGATAAAATCTCTGAGTCCAACCGGGCCCT CTGCTAACCATGTTTCATGCCTTCTTCTCTTCTCTAC AG
62	Envelope; VSV- G; Glycoprotein envelope-cell entry	ATGAAGTGCCTTTTGTACTTAGCCTTTTATTTCATT GGGGTGAATTGCAAGTTCACCATAGTTTTCACACA CAACCAAAAGGAACTGGAAAAATGTTCTTCTA ATTACCATATTGCCCCGTCAAGCTCAGATTTAAAT GGCATAATGACTTAATAGGCACAGCCTTACAAGTC AAAATGCCCAAGAGTCACAAGGCTATTCAAGCAG ACGGTTGGATGTGTCATGCTTCCAAATGGGTCACT ACTTGTGATTTCCGCTGGTATGGACCGAAGTATAT AACACATTCCATCCGATCCTTCACTCCATCTGTAGA ACAATGCAAGGAAAGCATTGAACAAACGAAACAA GGAACCTGGCTGAATCCAGGCTTCCCTCCTCAAAG TTGTGGATATGCAACTGTGACGGATGCCGAAGCAG TGATTGTCCAGGTGACTCCTCACCATGTGCTGGTTG ATGAATACACAGGAGATGGGTTGATTACAGTTT ATCAACGGAATGCAGCAATTACATATGCCCCAC TGTCATAACTCTACAACCTGGCATTCTGACTATAA GGTCAAAGGGCTATGTGATTCTAACCTCATTTCCAT GGACATCACCTTCTCTCAGAGGACGGAGAGCTAT CATCCCTGGGAAAGGAGGGCACAGGTTTCAAG TAACTACTTTGCTTATGAACTGGAGGCAAGGCCT GCAAAATGCAATACTGCAAGCATTGGGGAGTCAG ACTCCCATCAGGTGTCTGGTTCGAGATGGGTGATA AGGATCTCTTTGCTGCAGCCAGATTCCTGAATGC CCAGAAGGGTCAAGTATCTCTGCTCCATCTCAGAC CTCAGTGGATGTAAGTCTAATTCAGGACGTGAGA GGATCTTGATTATTCCCTCTGCCAAGAACTGG AGCAAAATCAGAGCGGGTCTTCCAATCTCTCCAGT GGATCTCAGCTATCTTGCTCTTAAACCCAGGAA CCGGTCTCTGCTTTCACCATAATCAATGGTACCCATA AATACTTTGAGACCAGATACATCAGAGTCGATATT GCTGCTCCAATCTCTCAAGATGGTCGGAATGAT CAGTGGAACTACCACAGAAAGGGAACGTGGGAT GACTGGGCACCATATGAAGACGTGGAATTTGGACC CAATGGAGTTCTGAGGACCAAGTTCAGGATATAAGT TTCCTTTATACATGATTGGACATGGTATGTTGGACT CCGATCTTCATCTTAGCTCAAAGGCTCAGGTGTTCTG AACATCCTCATTCAAGACGCTGCTTCGCAACTT CCTGATGATGAGGTTTATTTTTTGGTGATACTGGG CTATCCAAAAATCCAATCGAGCTTGTAAGGTTG GTTCAGTAGTTGGAAGCTCTATTGCCTCTTTTTT CTTTATCATAGGGTTAATCATTGGACTATTCTTGGT TCTCCGAGTTGGTATCCATCTTGCATTAAATTAAA GCACACCAAGAAAGACAGATTATACAGACATA GAGATGA
63	Envelope; Rabbit beta globin poly A; RNA stability	AGATCTTTTCCCTCTGCCAAAAATTATGGGGACAT CATGAAGCCCCTTGAGCATCTGACTTCTGGCTAAT AAAGGAAATTTATTTTCATTGCAATAGTGTGTTGG AATTTTTGTGTCTCTCACTCGGAAGGACATATGGG AGGGCAATCATTAAACATCAGAAAGAGTATT GGTTTAGAGTTTGGCAACATATGCCCATATGCTGG CTGCCATGAACAAAGGTTGGCTATAAAGAGGTCT CAGTATATGAACAGCCCCCTGCTGTCCATTCCCTTA TTCCATAGAAAAGCCTTGACTTGAGGTTAGATTTT TTTATATTTTGTGTTATTTTCTTTTCTTAAACAT

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SEQ ID NO:	Description	Sequence
		CCCTAAAATTTTCCTTACATGTTTACTAGCCAGAT TTTTCTCCTCTCCTGACTACTCCAGTCATAGCTG TCCCTCTTCTTATGGAGATC
64	Promoter; EF-1	CCGGTGCCTAGAGAAGGTGGCGCGGGTAAACTG GGAAAGTGATGTCTGTACTGGCTCCGCCTTTTCC CGAGGGTGGGGAGAACCCTATATAAGTGCAGTA GTCGCCGTGAACGTTCTTTTCGCAACGGGTTTGCC GCCAGAACACAGGTAAGTCCCGTGTGTGGTTCCCG CGGCCTGGCCTCTTACCGGTTATGGCCTTGCGT GCCTTGAATTACTTCCACGCCCTGGCTGCAGTAC GTGATTCTTGATCCCGAGCTTCGGGTGGAAGTGG GTGGGAGAGTTTCAGGCCTTGCCTTAAGGAGCCC CTTCGCCTCGTGTGAGTTGAGCCTGGCCTGGG CGCTGGGCGCGCGCTGCGAATCTGGTGGCACCT TCGCGCCTGTCTCGCTGCTTTCGATAAGTCTCTAGC CATTTAAAATTTTGTGATGACCTGCTGCGACGCTTTT TTTCTGGCAAGATAGTCTTGTAAATCGGGCCAAG ATCTGCACACTGGTATTTCGGTTTGGGGCCGCG GCGGCGACGGGGCCGTGCGTCCCAGCGCACATGT TCGGCGAGGCGGGCCTGCGAGCGCGGCCACCGA GAATCGGACGGGGTAGTCTCAAGCTGGCCGCCCT GCTCTGGTGCCTGGCCTCGCGCCCGCTGTATCGC CCCGCCTTGGGCGCAAGGCTGGCCCGTCCGCGAC CAGTTGCGTGAGCGGAAGATGGCGCTTCCCGGC CCTGCTGCAGGAGCTCAAAATGGAGGACGCGGC GCTCGGAGAGCGGGCGGTGAGTCAACCACACA AAGGAAAAGGCCTTTCGCTCTCAGCCGTGCTT CATGTGACTCCACGGAGTACCGGGCGCGTCCAGG CACCTCGATTAGTTCTCGAGCTTTTGGAGTACGTG TCTTTAGGTTGGGGGAGGGTTTATGCGATGGA GTTTCCCACTGAGTGGTGGAGACTGAAGTTA GGCCAGCTTGGCACTTGATGTAATCTCCTTGGAAT TTGCCCTTTTGTGAGTTGGATCTTGGTTCATTCTCA AGCCTCAGACAGTGGTTCAAAGTTTTTCTTCCAT TTCAGGTGTCGTGA
65	Promoter; PGK	GGGGTTGGGGTGGCGCTTTTCCAAGCAGCCCTG GGTTTGGCAGGGACGCGGTGCTTGGGCGTGGT TCCGGAAACGCAGCGCGCCGACCTGGGTCTCG CACATTCTTACGTCCTGTCGAGCGTCACCCGGAT CTTCGCGCTACCTTGTGGGCCCCCGGCGACGC TTCTGTCTCGCCCCTAAGTCGGGAAGTTCTTGC GGTTCGCGCGTGGCGGACGTGACAAACGGAAGC CGCACGTCTACTAGTACCCTCGCAGACGGACAGC GCCAGGGAGCAATGGCAGCGCGCCGACCGCATG GGCTGTGGCCAATAGCGGCTGCTCAGCAGGCGCG CCGAGAGCAGCGCGCGGAAGGGCGGTGCGGGA GGCGGGTGTGGGCGGTAGTGTGGCCCTGTTC TGCCCGCGCGTGTTCGCAATCTGCAAGCCTCCG GAGCGACGTGCGCAGTCGGCTCCCTCGTTGACCG AATCACCGACCTCTCTCCCCAG
66	Promoter; UbC	GCGCCGGGTTTGGCGCTCCCGCGGGCGCCCCC TCCTCACGGCGAGCGCTGCCAGTCAGACGAAGG CGCAGGAGCGTTCTGATCTTCCGCCCGGACGCT CAGGACAGCGCCCGCTGCTCATAAGACTCGGCCT TAGAACCACAGTATCAGCAGAAGGACATTTAGGA CGGGACTTGGTGACTCTAGGGCACTGGTTTCTTT CCAGAGAGCGGAACAGGCGAGGAAAGTAGTCCC TTCTCGCGATCTTTCGCGAGGATCTCCTGGGGC GGTGAACGCCGATGATTATATAAGGACGCGCCGG TGTGGCACAGCTAGTTCCGTGCGAGCGGGATTTG GGTCGCGTTCTTGTGTTGGATCGCTGTGATCGTC ACTTGGTGAGTTGCGGCTGCTGGGCTGGCCGGGG CTTTCGTGGCGCGCGGCGCTCGGTGGGACGGAA GCGTGTGGAGAGACCGCAAGGGCTGTAGTCTGGG TCCGCGAGCAAGGTTGCCCTGAAGTGGGGTTGGG GGGAGCGCACAAAATGGCGCTGTTCAGTCTT GAATGGAAGACGCTTGAAGCGGGCTGTGAGGTC GTTGAAACAAGGTGGGGGCGATGGTGGCGGCAA GAACCCAAGGCTTGGAGCCTTCGCTAATGCGGGA AAGCTCTTATTCGGGTGAGATGGGCTGGGGACCA TCTGGGACCTTGACGTGAAGTTTGTCACTGACTG GAGAATCGGGTTTGTGCTGCGTTGCGGGGCGG CAGTTATGCGGTGCCGTTGGGCAGTGACCCGTAC

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SEQ ID NO:	Description	Sequence
		CTTTGGGAGCGCGCCCTCGTCGTGTCGTGACGTC ACCCGTTCTGTTGGCTTATAATGCAGGGTGGGGCC ACCTGCCGGTAGGTGTGCGGTAGGCTTTCTCCGTC GCAGGACGCAGGGTTCGGGCCTAGGGTAGGCTCTC CTGAATCGACAGGCGCCGACCTCTGGTGAGGGGA GGGATAAGTGAGGCGTCAGTTTCTTTGGTCGGTTTT ATGTACCTATCTTCTTAAGTAGCTGAAGCTCCGGTT TTGAACTATGCGCTCGGGTTGGCGAGTGTGTTTT GTGAAGTTTTTTAGGCACCTTTGAAATGTAATCAT TTGGGTCAATATGTAATTTTCAGTGTAGACTAGTA AA
67	Poly A; SV40	GTTTATTGCAGCTTATAATGGTTACAAATAAAGCA ATAGCATCACAAATTCACAAATAAAGCATTTTTTT CACTGCATTCTAGTTGTGGTTTGTCCAACTCATCA ATGTATCTTATCA
68	Poly A; bGH	GACTGTGCCTTCTAGTTGCCAGCCATCTGTTGTTTG CCCCCCCCGTGCCTTCTTGACCTGGAAGGTGC CACTCCCACTGTCCTTTCCTAATAAAATGAGGAAA TTGCATCGCATTGTCTGAGTAGGTGTCTATTTCT TGGGGGTGGGTGGGCAGGACAGCAAGGGGA GGATTGGGAAGACAATAGCAGGCATGCTGGGGAT GCGGTGGGCTCTATGG
69	HIV Gag; Bal	ATGGGTGCGAGAGCGTCAGTATTAAGCGGGGAG AATTAGATAGGTGGGAAAAAATTCGGTTAAGGCCA GGGGAAAGAAAAAATATAGATTAAACATATAG TATGGGCAAGCAGGGAAGTAAAGATTTCGAGT CAATCTGGCCTGTAGAAACATCAGAAGGCTGCA GACAAATACTGGGACAGCTACAACCATCCCTTCAG ACAGGATCAGAAGAACTTAGATCATTATATAATAC AGTAGCAACCTCTATTGTGTACATCAAAAGATAG AGGTAAAGACACCAAGGAAGCTTAGACAAAAT AGAGGAAGAGCAAAACAAATGTAAGAAAAAGGCA CAGCAAGCAGCAGCTGACACAGGAAACAGCGGTC AGGTCAGCCAAAATTTCCCTATAGTGCAAGCTC CAGGGGCAAAATGGTACATCAGGCCATATCACCTAG AACTTTAAATGCATGGGTAAAGTAATAGAAAGAG AAAGCTTTCAGCCAGAAGTAATACCATGTTTTTC AGCATTATCAGAAGGAGCCACCCACAAGATTTAA ACACCATGCTAAACACAGTGGGGGACATCAAGC AGCCATGCAAAATGTTAAAGAACCCATCAATGAGG AAGCTGCAAGATGGGATAGATTGCATCCCGTGCAG GCAGGGCCTGTGCAACAGGCCAGATAAGAGATCC AAGGGGAAGTGACATAGCAGGAAGTACCAGTACC CTTCAGGAACAAATAGGATGGATGACAAGTAATCC ACCTATCCAGTAGGAGAAATCTATAAAAGATGGA TAATCTGGGATTAATAAAATAGTAAGGATGTAT AGCCCTACCAGCATTTTGGACATAAGACAAGGACC AAAGGAACCTTTTAGAGACTATGTAGACCGGTTCT ATAAACTCTAAGAGCCGAGCAAGCTTCACAGGA GGTAAAAAATGGATGACAGAAACCTTGTGGTCC AAAATGCGAAGCCAGATTGTAAGACTATTTAAAA GCATTGGGACCAGCAGCTACACTAGAAGAAATGAT GACAGCATGTCAGGGAGTGGGAGGACCCAGCCAT AAAGCAAGAAATTTGGCAGAAGCAATGAGCCAAG TAACAAATTCAGCTACCATAATGATGCAGAAAGGC AATTTTAGGAACCAAGAAAGATTGTTAAATGTTT CAATTGTGGCAAGGAAGGGCAGATAGCCAGAAAC TGCAAGGCCCCTAGGAAAAGGGGCTGTGGAAAT GTGGAAGGAAGGACACCAATGAAAGACTGTAC TGAGAGACAGGCTAATTTTTTAGGGAAGATCTGGC CTTCCCAAGGAAGGCCAGGGAATTTCTTCAG AGCAGACCAGAGCCAACAGCCCCACGCCCCAC CAGAAGAGAGCTTCAGGTTTGGGAAGAGACAAC AACTCCCTCTCAGAAGCAGGAGCTGATAGACAAGG AACTGTATCCTTTAGCTTCCCTCAGATCACTCTTGG GCAACGACCCCTCGTCACAATAA
70	HIV Pol; Bal	ATGAATTGCGAGGAAGATGGAAACCAAAATGA TAGGGGAATTTGGAGTTTTATCAAAGTAAGACAG TATGATCAGATACATAGAAATCTGTGGACATAA AGCTATAGGTACAGTATTAATAGGACCTACACCTG TCAACATAATTGGAAGAAATCTGTTGACTCAGATT GGTTGCACTTTAAATTTCCCATTAGTCTATTGAA

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SEQ ID NO:	Description	Sequence
		ACTGTACCAGTAAAATTAAAACCAGGAATGGATGG CCCAAAAGTTAAACAATGGCCACTGACAGAAGAA AAAATAAAAGCATTAAATGGAAATCTGTACAGAAAT GGAAAAGGAAGGGAAAATTTCAAAAATTGGGCCT GAAAAATCCATACAATACTCCAGTATTTGCCATAAA GAAAAAGACAGTACTAAATGGAGAAAATTAGTA GATTTAGAGAACTTAATAAGAAAACCTCAAGACTT CTGGGAAGTACAATTAGGAATACACATCCCGCAGG GGTAAAAAAGAAAAATCAGTAACAGTACTGGA TGTGGGTGATGCATATTTTCAGTTCCCTTAGATAA AGAATTCAGGAAGTATACTGCATTACCATACCTA GTATAACAATGAAACACCAGGGATCAGATATCA GTACAATGTACTTCCACAGGGATGGAAGGATCAC CAGCAATATTTCAAAGTAGCATGACAAGAACTTTA GAGCCTTTTAGAAAACAAAATCCAGAAATAGTGAT CTATCAATACATGGATGATTTGTATGTAGGATCTG ACTTAGAAATAGGGCAGCATAGAACAAAATAGA GGAAGTGAAGCAACATCTGTTGAGGTGGGGATTTA CCACACCAGACAAAAACATCAGAAAGAACCTCC ATTCCTTTGGATGGGTTATGAACTCCATCCTGATAA ATGGACAGTACAGCCTATAGTGCTGCCAGAAAAAG ACAGCTGGACTGTCAATGACATACAGAAGTTAGTG GGAAAATTGAATTGGGCAAGTCAGATTTACCCAGG AATTAAAGTAAAGCAATATGTAGGCTCCTTAGGG GAACCAAGGCATTAACAGAAGTAATACCACTAAC AAAAGAAACAGAGCTAGAACTGGCAGAGAACAGG GAAATTTAAAAGAACCAAGTACATGGGGTGTATTA TGACCCATCAAAAGACTTAATAGCAGAAATACAGA AGCAGGGGCAAGGCCAATGGACATATCAAAATTTAT CAAGAGCCATTTAAAAATCTGAAACAGGAAAAAT ATGCAAGAATGAGGGGTGCCACACTAATGATGTA AAACAATTAAACAGAGGCAGTGCAAAAATAACCA CAGAAAGCATAGTAATATGGGGAAAGACTCCTAA ATTTAAACTACCCATACAAAAGAAACATGGGAA ACATGGTGGACAGAGTATTGGCAAGCCACCTGGAT TCCTGAGTGGGAGTTTGTCAATACCCCTCCCTTAGT GAAATTATGTTACAGTTAGAGAAAGAACCCATAA TAGGAGCAGAAACATTCTATGTAGATGGAGCAGCT AACCGGGAGACTAAATTAGGAAAAGCAGGATATG TTACTAACAGAGGAAGACAAAAGTTGTCTCCCTA ACTGACACAACAAATCAGAAGACTGAGTTACAAG CAATTCATCTAGCTTTACAAGATTGAGGATTAGAA GTAAACATAGTAACAGACTCACAATATGCATTAGG AATCATTAAGCACAACAGATAAAAGTGAATCAG AGTTAGTCAGTCAAATAATAGAACAGTTAATAAAA AAGGAAAAGGTCTACCTGGCATGGGTACCAAGCGC ACAAGGAATTGGAGGAAATGAACAAGTAGATAA ATTAGTCAGTACTGGAATCAGGAAAGTACTA
71	HIV Integrase; Ba1	TTTTATAGTGAATAGATATAGCCCAAGAAGAACA TGAGAAATATCACAGTAATGGAGAGCAATGGCTA GTGATTTTAACCTGCCACCTGTGGTAGCAAAAGAA ATAGTAGCCAGCTGTGATAAATGTCAGCTAAAAGG AGAAGCCATGCATGGACAAGTAGACTGTAGTCCAG GAATATGGCACTAGATTGTACACATTTAGAAGGA AAAATTATCCTGGTAGCAGTTTATGTAGCCAGTGG ATATATAGAAGCAGAAGTTATTCCAGCAGAGACAG GGCAGGAAACAGCATACTTTCTCTTAAATTAGCA GGAAGATGGCCAGTAAAAACAATACATACAGACA ATGGCAGCAATTTCACTAGTACTACAGTCAAGGCC GCCTGTTGGTGGGCGGGGATCAAGCAGGAATTTGG CATTCCCTACAATCCCCAAAGTCAGGGAGTAGTAG AATCTATAAATAAAGAAATTAAGGAAATTTAGGA CAGGTAAGAGATCAGGCTGAACATCTTAAACAGC AGTACAAATGGCAGTATTCATCCACAATTTAAAA GAAAAGGGGGGATTGGGGGGTATAGTGCAGGGGA AAGAATAGTAGACATAATAGCAACAGACATACAA ACTAAAGAATTACAAAACAAATACAAAAATTC AAATTTTCGGGTTTATTACAGGGACAGCAGAGATC CACTTTGGAAGGACAGCAAGCTTCTCTGGAAA GGTGAAGGGGAGTAGTAATACAAGATAATAGTG ACATAAAAGTAGTACCAAGAAGAAAAGCAAGAT CATTAGGATTATGGAACACAGATGGCAGGTGATG ATGTGTGGCAAGTAGACAGGATGAGGATTAG

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SEQ ID NO:	Description	Sequence
72	Envelope; RD114	<p> ATGAAACTCCCAACAGGAATGGTCATTTTATGTAG CCTAATAATAGTTCGGGCAGGGTTTGACGACCCCC GCAAGGCTATCGCATTAGTACAAAAACAACATGGT AAACCATGCGAATGCAGCGGAGGGCAGGTATCCG AGGCCCCACCGAACTCCATCCAACAGGTAACCTGTC CCAGGCAAGACGGCTACTTAATGACCAACCAAAA ATGGAAATGCAGAGTCACTCCAAAAATCTCAGCC CTAGCGGGGAGAACTCCAGAACTGCCCTGTAAAC ACTTTCAGGACTCGATGCACAGTTCTTGTTATACT GAATACCGGCAATGCAGGGCGAATAATAAGACAT ACTACACGGCCACCTTGCTTAAATACGGTCTGGG AGCCTCAACGAGGTACAGATATTACAAAACCCCAA TCAGCTCCTACAGTCCCCTTGAGGGCTCTATAA ATCAGCCCCGTTTGCTGGAGTGCCACAGCCCCCATC CATATCTCCGATGGTGGAGGCCCTCGATACTAA GAGAGTGTGGACAGTCCAAAAAGGCTAGAACAA ATTCATAAGGCTATGCATCTGAACTTCAATACCA CCCCTTAGCCCTGCCAAAGTCAGAGATGACCTTA GCCTTGATGCACGGACTTTTGATATCCTGAATACC ACTTTTAGGTTACTCCAGATGTCCAATTTAGCCCT GCCCAAGATTGTTGGCTCTGTTAAACTAGGTAC CCCTACCCCTCTTGCGATACCCACTCCCTCTTAAAC CTACTCCCTAGCAGACTCCCTAGCGAATGCCTCCT GTCAGATTATACCTCCCTCTTGTTCAACCGATGC AGTTCTCCAACCTCGTCTGTTTATCTTCCCCTTTCAT TAACGATACGGAAACAAATAGACTTAGGTGCAGTCA CCTTTACTAAGTGCACCTCTGTAGCCAATGTCAGTA GTCCTTTATGTGCCCTAAACGGGTCACTCTCCTCT GTGGAAATAACATGGCATACACCTATTACCCCAA AACTGGACAGGACTTTGCGTCCAAGCCTCCCTCCT CCCCACATTGACATCATCCCGGGGATGAGCCAG TCCCCATTCCTGCCATTGATCATTATATACATAGAC CTAACGAGCTGTACAGTTTATCCCTTACTAGCTG GACTGGGAATCACCGCAGCATTCACCACCGAGCT ACAGGCCCTAGGTGTCTCCGTCAACCAGTATACAAA ATTATCCCATCAGTTAATATCTGATGTCCAAGTCTT ATCCGGTACCATAACAAGATTACAAAGACCAGGTAG ACTCGTTAGCTGAAGTAGTTCTCCAAAATAGGAGG GGACTGGACCTACTAACGGCAGAACAGGAGGAA TTTGTTTAGCCTTACAAGAAAAATGCTGTTTTATG CTAACAAGTCAGGAATTGTGAGAAACAAAAAAG AACCTACAAGAGAATTACAAAACCGAGGGAA AGCCTGGCATCCAACCTCTCTGGACCGGGCTGCA GGGCTTTCTTCCGTACCTCCTACCTCTCCTGGGACC CCTACTCACCTCCTACTACTAATAACATTGGGCC ATGCGTTTTCAATCGATTGGTCCAATTTGTTAAAGA CAGGATCTCAGTGGTCCAGGCTCTGGTTTTGACTC AGCAATATCACCAGCTAAACCCATAGAGTACGAG CCATGA </p>
73	Envelope; GALV	<p> ATGCTTCTCACCTCAAGCCCGACACCTTCGGCA CCAGATGAGTCCTGGGAGCTGGAAAAGACTGATCA TCCTCTTAAGCTGCGTATTCGGAGACGGCAAAACG AGTCTGCAGAAATAAGAACCACCAGCCTGTGAC CCTCACCTGGCAGGTACTGTCCAAACTGGGGACG TTGTCTGGGACAAAAGGCAGTCCAGCCCCCTTGG ACTTGGTGGCCCTCTCTTACACCTGATGTATGTGCC CTGGCGGCCGCTCTGAGTCTGGGATATCCCGGG ATCCGATGTATCGTCTCTAAAAGAGTTAGACCTC CTGATTGAGACTATACTGCGCTTATAAGCAAATC ACCTGGGGAGCCATAGGGTGCAGCTACCCCTCGGGC TAGGACCAGGATGGCAAATTCCTCTTCTACGTGT GTCCCCGAGCTGGCCGAACCAATTCAGAAGCTAGG AGGTGTGGGGGGCTAGAATCCCTACTGTAAAGA ATGGAGTTGTGAGACCACGGGTACCGTTTATTGGC AACCCAAGTCTCATGGGACCTCATACTGTAAAA TGGGACCAAAATGTGAATGGGAGCAAAAATTTC AAAAGTGTGAACAAACCGGCTGGTGTAAACCCCTC AAGATAGACTTCACAGAAAAAGGAAACTCTCCA GAGATTGGATAACGGAAAAAACCTGGGAATTAAG GTTCTATGTATATGGACACCCAGGCATACAGTTGA CTATCCGCTTAGAGGTCACTAACATGCCGTTGTG GCAGTGGGCCAGACCTGTCTTGGCGAACAGGG ACCTCTAGCAAGCCCCCTCACTCTCCCTCTCTCCCC ACGGAAAGCGCGCCACCCCTCTACCCCGGCGG </p>

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SEQ ID NO:	Description	Sequence
		CTAGTGAGCAAACCCCTGCGGTGCATGGAGAACT GTTACCCCTAAACTCTCCGCCTCCACCAAGTGGCGA CCGACTCTTTGGCCTTGTGCAGGGGGCCTTCCTAAC CTTGAATGCTACCAACCCAGGGGCCACTAAGTCTT GCTGGCTCTGTTTGGGCATGAGCCCCCTTATTATG AAGGGATAGCCTCTTCAGGAGAGGTGCGTTATACC TCCAACCATACCCGATGCCACTGGGGGGCCCAAGG AAAGCTTACCCTCACTGAGGTCTCCGGACTCGGGT CATGCATAGGGAAGGTGCCTCTTACCCATCAACAT CTTTGCACCCAGACCTTACCCATCAATTCTCTAAA AACCATCAGTATCTGCTCCCCCTCAAACCATAGCTG GTGGGCCCTGCAGCACTGGCCTCACCCCTGCCTCT CCACCTCAGTTTTTAATCAGTCTAAAGACTTCTGTG TCCAGGTCCAGCTGATCCCCGCATCTATTACCATT CTGAAGAAACCTTGTTACAAGCCTATGACAAATCA CCCCCAGGTTTAAAAGAGAGCCTGCCCTACTTAC CCTAGCTGTCTTCTGGGGTTAGGGATTGCGGCAG GTATAGGTACTGGCTCAACCGCCCTAATTAAAGGG CCCATAGACCTCCAGCAAGGCCTAACAGCCTCCA AATCGCCATTGACGCTGACCTCCGGGCCCTTCAGG ACTCAATCAGCAAGCTAGAGGACTCACTGACTTCC CTATCTGAGGTAGTACTCCAAAATAGGAGAGGCCCT TGACTTACTATTCCTTAAAGAAGGAGGCCCTCTGCG CGGCCCTAAAAGAAGAGTGTGTTTTTATGTAGAC CACTCAGGTGCAGTACGAGACTCCATGAAAAAAT TAAAGAAAGACTAGATAAAAGACAGTTAGAGCGC CAGAAAAACCAAACTGGTATGAAGGGTGGTTCA ATAACTCCCTTGGTTTACTACCTACTATCAACCA TCGCTGGGCCCTATTGCTCCTCCTTTTGTACTCA CTCTTGGGCCCTGCATCATCAATAAATTAATCCAAT TCATCAATGATAGGATAAGTGCAGTCAAAATTTTA GTCCTTAGACAGAAATATCAGACCTTAGATAACGA GGAAACCTTTAA
74	Envelope; FUG	ATGGTTCCGCAGGTTCTTTTGTGTACTCCTTCTG GGTTTTTCTGTTGTGTTTCGGGAAGTTCCTCATTTAC ACGATACCAGACGAACTTGGTCCCTGGAGCCCTAT TGACATACACCATCTCAGCTGTCCAAATAACCTGG TTGTGGAGGATGAAGGATGTACCAACCTGTCCGAG TTCTCCTACATGGAACCTCAAAGTGGGATACATCTC AGCCATCAAAGTGAAACGGGTTCACTTGACACAGGTG TTGTGACAGAGGCAGAGACTACACCAACTTTGTT GGTTATGTCAACAACCATTCAGAGAAAGCATT CCGCCCCACCCAGACGCATGTAGAGCCGCTATA ACTGGAAGATGGCCGGTGACCCAGATATGAAGA GTCCTTACACAATCCATACCCGACTACCACTGGC TTCGAACGTGAAGAACCAAGAGTCCCTCATT ATCATATCCCCAAGTGTGACAGATTTGGACCATA TGACAAATCCCTTCACTCAAGGGTCTTCCCTGGCG GAAAGTGCTCAGGAATAACGGTGTCTCTACCTAC TGCTCAACTAACCATGATTACACCATTTGGATGCC CGAGAATCCGAGACCAAGGACACCTTGTGACATT TTACCAATAGCAGAGGGAAGAGCATCCAACGG GAACAAGACTTGCGGCTTTGTGGATGAAAGAGGCC TGTATAAGTCTCTAAAAGGAGCATGCAGGCTCAAG TTATGTGGAGTTCTTGGACTTAGACTTATGGATGG AACATGGGTGCGATGCAACATCAGATGAGACC AAATGGTGGCCTCCAGATCAGTTGGTGAATTTGCA CGACTTTCGCTCAGACGAGATCGAGCATCTCGTTG TGGAGGAGTTAGTTAAGAAAAGAGAGGAATGTCT GGATGCATTAGAGTCCATCATGACCACCAAGTCAG TAAGTTTCAGACGTCTCAGTCACCTGAGAAAATTT GTCCAGGGTTTGAAAAGCATATACCATATTCAA CAAACCTTGATGGAGGCTGATGCTCACTACAAGT CAGTCCGACCTGGAATGAGATCATCCCTCAAAA GGGTGTGTGAAGTTGGAGGAAGGTGCCATCCTCA TGTGAACGGGGTGTTCATGATGATATAATATTAG GGCTGACGACCATGTCTAATCCAGAGATGCAA TCATCCCTCCTCCAGCAACATATGGAGTTGTGGGA ATCTTCAGTTATCCCCCTGATGCACCCCTGGCAGA CCCTTCTACAGTTTTCAAAGAAGGTGATGAGGCTG AGGATTTTGTGAAGTTCACCTCCCCGATGTGTACA AACAGATCTCAGGGGTTGACCTGGGTCTCCGAAC TGGGGAAGATATGATTGATGACTGCAGGGGCCAT GATTGGCCTGGTGTGATATTTCCCTAATGACATG GTGCAGAGTTGGTATCCATCTTGCATTAAATTAA

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SEQ ID NO:	Description	Sequence
		AGCACACCAAGAAAAGACAGATTTATACAGACAT AGAGATGAACCGACTTGGAAGTAA
75	Envelope; LCMV	ATGGGTGAGATTGTGACAATGTTTGAGGCTCTGCC TCACATCATCGATGAGGTGATCAACATTGTCTATTA TTGTGCTTATCGTGATCAGGGTATCAAGGCTGTCT ACAATTTTGCCACCTGTGGGATATTCGCATTGATCA GTTTCCTACTTCTGGCTGGCAGGTCTGTGGCATGT ACGGTCTTAAGGGACCCGACATTTACAAAGGAGTT TACCAATTAAAGTCAGTGGAGTTTGATATGTCACA TCTGAACCTGACCATGCCCAACGCATGTTTCAGCCA ACAACCTCCACCATTACATCAGTATGGGGAATTCT GGACTAGAATTGACCTTCACCAATGATTCATCAT CAGTCACAACCTTTGCAATCTGACCTCTGCCTTCAA CAAAAAGACCTTTGACCACACACTCATGAGTATAG TTTCGAGCCTACACCTCAGTATCAGAGGGAATCC AACTATAAGGCAGTATCCTGCGACTTCAACAATGG CATAACCATCCAATACAACCTTGACATTCAGATC GACAAAGTGCTCAGAGCCAGTGTAGAACCTTCAGA GGTAGAGTCTTAGATATGTTTAGAACTGCCTTCGG GGGGAATACATGAGGAGTGGCTGGGGCTGGACA GGCTCAGATGGCAAGACCACCTGGTGTAGCCAGAC GAGTTACCAATACCTGATTATACAAAATAGAACCT GGGAAAACCACTGCACATATGCAGGTCTTTTGGG ATGTCCAGGATTCTCCTTTCCCAAGAGAAGACTAA GTTCTTCACTAGGAGACTAGCGGCACATTACCT GGACTTTGTGAGACTCTTCAGGGGTGGGAATCCA GGTGGTTATTGCCTGACCAATGGATGATTCTTGCT GCAGAGCTTAAGTGTTCGGGAACACAGCAGTTGC GAAATGCAATGTAAATCATGATGCCGAATTCTGTG ACATGCTGCGACTAATTGACTACAACAAGCTGCT TTGAGTAAGTTCAAAAGAGGACGTAGAATCTGCCTT GCACCTTATTCAAAACAACAGTGAATCTTTGATTTC AGATCAACTACTGATGAGGAACCACTTGAGAGATC TGATGGGGTGCCATATTGCAATTACTCAAAGTTT TGGTACCTAGAACATGCAAGACCGGCGAACTA GTGTCCCAAGTGCTGGCTTGTCACCAATGGTTCTT ACTTAAATGAGACCCACTTCAGTGATCAAAATCGAA CAGGAAGCCGATAACATGATTACAGAGATGTTGAG GAAGGATTACATAAAGAGGCAGGGGAGTACCCCT CTAGCATTGATGGACCTTCTGATGTTTTCCACATCT GCATATCTAGTCAGCATCTTCTGCACCTTGTCAAA ATACCAACACACAGGCACATAAAGGTGGCTCATG TCCAAAGCCACACCGATTAAACCAAAAGGAATTT GTAGTTGTGGTGCATTTAAGGTGCCTGGTGTAAAA ACCGTCTGGAAGGACGCTGA
76	Envelope; FPV	ATGAACACTCAAACTCCTGGTTTTTCGCCCTTGTGGCA GTCATCCCCACAAATGCAGACAAAATTTGTCTTGG ACATCATGCTGTATCAAAATGGCACCAGTAACACA CACTCACTGAGAGAGGAGTAGAAGTTGTCAATGCA ACGGAAACAGTGGAGCGGACAAACATCCCCAAAA TTTGCTCAAAAGGGAAAAGAACCACTGATCTTGGC CAATGCGGACTGTTAGGGACCAATTACCGGACCACT TCAATGCGACCAATTTCTAGAATTTTCAGCTGATCT AATAATCGAGAGACGAGAAGGAAATGATGTTTGT ACCCGGGGAAGTTTGTAAATGAAGAGGCATTGCGA CAAATCCTCAGAGGATCAGGTGGGATTGACAAAG AAACAATGGGATTCACATATAGTGAATAAGGACC AACGGAACAACACTAGTGATGTAGAAGATCAGGGT CTTCATTCTATGCAGAAATGGAGTGGCTCCTGTCA AATACAGACAATGCTGCTTTCCCAAAATGACAAA ATCATACAAAACACAAGGAGAGAATCAGCTCTG ATAGTCTGGGGAATCCACCATTGAGGATCAACCAC CGAACAGACCAAACTATATGGGAGTGGAAATAAA CTGATAACAGTCGGGAGTTCCAAATATCATCAATC TTTGTGCGGAGTCCAGGAACACGACCGCAGATAA ATGGCCAGTCCGGACGGATTGATTTTCATTGGTTG ATCTTGGATCCCAATGATACAGTTACTTTTAGTTTC AATGGGGCTTTTATAGTCCAAATCGTGCCAGCTT CTTGAGGGGAAAGTCCATGGGGATCCAGAGCGAT GTGCAGGTGATGCCAATTGCGAAGGGGAATGCTA CCACAGTGGAGGGACTATAACAAGCAGATTGCCTT TTCAAAACATCAATAGCAGAGCAGTTGGCAAATGC CCAAGATATGTAAACAGGAAAGTTATATTGGC AACTGGGATGAAGAACGTTCCCGAACCTTCCAAAA

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SEQ ID NO:	Description	Sequence
		AAAGGAAAAAAGAGGCCTGTTTGGCGCTATAGC AGGGTTTATTGAAAATGGTTGGGAAGGTCTGGTCG ACGGGTGGTACGGTTTCAGGCATCAGAATGCACAA GGAGAAGGAACGAGCAGACTACAAAAGCACCC AATCGGCAATTGATCAGATAACCGAAAGTTAAAT AGACTCATTGAGAAAACCAACGAGCAATTTGAGCT AATAGATAATGAATTCAGTGGTGGAAAAGCAG ATTGGCAATTTAATTAAGTGGACCAAGACTCCAT CACAGAAGTATGGTCTTACAATGCTGAACCTCTTG TGGCAATGGAAAACGAGCACACTATTGATTTGGCT GATTCAGAGATGAACAAGCTGTATGAGCGAGTGA GGAAACAATTAAAGGAAAATGCTGAAGAGGATGG CACTGGTTGCTTTGAAATTTTTCATAATGTGACGA TGATTGTATGGCTAGTATAAGGAACAATACTTATG ATCAGAGCAATAAGAGAAAGCGATGCAAAA TAGAATACAAATTGACCCAGTCAAATTGAGTAGTG GCTACAAAGATGTGATACTTTGGTTTAGCTTCGGG GCATCATGCTTTTTGCTCTTGGCATTGCAATGGGG CTTGTTTTCATATGTGTGAAGAACGGAACATGCG GTGCACTATTTGTATATAA
77	Envelope; RRV	AGTGTAACAGAGCACTTTAATGTGTATAAGGCTAC TAGACCATACCTAGCACATTGCGCCGATTGCGGGG ACGGGTACTTCTGCTATAGCCAGTTGCTATCGAG GAGATCCGAGATGAGGCGTCTGATGGCATGCTTAA GATCCAAGTCTCCGCCCAAATAGGTCTGGACAAGG CAGGCACCCACGCCACACGAAGCTCCGATATATG GCTGGTCATGATGTTGAGGAATCTAAGAGAGATTCT CTTGAGGGGTACACGTCGCGAGCGTGCTCCATAC ATGGGACGATGGGACACTTCATCGTCGCACACTGT CCACCAGGCGACTACCTCAAGGTTTCGTTGAGGA CGCAGATTGCGACGTGAAGGCATGTAAGGTCCAAT ACAAGCACAATCCATTGCGGTTGGGTAGAGAGAA GTTCTGGTTAGACCACACTTTGGCGTAGAGCTGC CATGCACCTCATACAGCTGACAACGGCTCCCACC GACGAGGAGATTGACATGCATACCCGCCAGATAT ACCGGATCGCACCCCTGCTATCACAGACGGCGGCA ACGTCAAATAACAGCAGGCGGCAGGACTATCAG GTACAACGTGACCTGCGGCGGTGACAACGTAGGCA CTACCAGTACTGACAAGACCATCAACACATGCAAG ATTGACCAATGCCATGCTGCCGTACCAGCCATGA CAAATGGCAATTTACCTCTCCATTGTTCCAGGGC TGATCAGACAGCTAGGAAAGGCAAGGTACACGTTCT CGTTCCCTCTGACTAACGTACCTGCGAGTGCCGTT TGGCTCGAGCGCCGGATGCCACCTATGGTAAGAAG GAGGTGACCCCTGAGATTACACCCAGATCATCCGAC GCTCTTCTCTATAGGAGTTTAGGAGCCGAACCGC ACCCGTACGAGGAATGGGTTGACAAGTTCTCTGAG CGCATCATCCAGTGACGGAAGAAGGATTGAGTA CCAGTGGGGCAACAACCCGCGGTCTGCCTGTGGG CGCAACTGACGACCGAGGGCAACCCCATGGCTG GCCACATGAAATCATTCAGTACTATTATGGACTAT ACCCCGCGCCACTATTGCCGCGAGTATCCGGGGCG AGTCTGATGGCCCTCCTAACTCTGGCGGCCACATG CTGCATGCTGGCCACCGCAGGAGAAAGTGCCTAA CACCGTACGCCCTGACGCCAGGAGCGGTGTTACCG TTGACACTGGGGCTGCTTGTGTCGCACCGAGGGC GAATGCA
78	Envelope; MLV 10A1	AGTGTAACAGAGCACTTTAATGTGTATAAGGCTAC TAGACCATACCTAGCACATTGCGCCGATTGCGGGG ACGGGTACTTCTGCTATAGCCAGTTGCTATCGAG GAGATCCGAGATGAGGCGTCTGATGGCATGCTTAA GATCCAAGTCTCCGCCCAAATAGGTCTGGACAAGG CAGGCACCCACGCCACACGAAGCTCCGATATATG GCTGGTCATGATGTTGAGGAATCTAAGAGAGATTCT CTTGAGGGGTACACGTCGCGAGCGTGCTCCATAC ATGGGACGATGGGACACTTCATCGTCGCACACTGT CCACCAGGCGACTACCTCAAGGTTTCGTTGAGGA CGCAGATTGCGACGTGAAGGCATGTAAGGTCCAAT ACAAGCACAATCCATTGCGGTTGGGTAGAGAGAA GTTCTGGTTAGACCACACTTTGGCGTAGAGCTGC CATGCACCTCATACAGCTGACAACGGCTCCCACC GACGAGGAGATTGACATGCATACCCGCCAGATAT ACCGGATCGCACCCCTGCTATCACAGACGGCGGCA ACGTCAAATAACAGCAGGCGGCAGGACTATCAG

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SEQ ID NO:	Description	Sequence
		GTACAACGTGTACCTGCGGCCGTGACAACGTAGGCA CTACCACTACTGACAAGACCATCAACACATGCAAG ATTGACCAATGCCATGCTGCCGTACCCAGCCATGA CAAATGGCAATTACCTCTCCATTGTTCCAGGGC TGATCAGACAGCTAGGAAAGGCAAGGTACACGTTT CGTTCCCTCTGACTAACGTACCTGCCGAGTGCCGT TGGCTCGAGCGCCGGATGCCACCTATGGTAAGAAG GAGGTGACCCCTGAGATTACACCCAGATCATCCGAC GCTCTTCTCTATAGGAGTTTAGGAGCCGAACCGC ACCCGTACGAGGAATGGGTGACAAGTTCTCTGAG CGCATCATCCAGTGACGGAAGAAGGGATTGAGTA CCAGTGGGGCAACAACCCGCCGCTGCTGCTGGG CGCAACTGACGACCGAGGGCAACCCCATGGCTG GCCACATGAAATCATTTCAGTACTATTATGGACTAT ACCCCGCCGCCACTATTGCCGCGAGTATCCGGGGCG AGTCTGATGGCCCTCCTAACTCTGCGGCCACATG CTGCATGCTGGCCACCGCGAGGAGAAAGTGCCATA CACCGTACGCCCTGACGCCAGGAGCGGTGGTACCG TTGACACTGGGGCTGCTTTGCTGCGCACCAGGGC GAATGCA
79	Envelope; Ebola	ATGGGTGTTACAGGAATATTGCAGTTACCTCGTGA TCGATTCAAGAGGACATCATTCTTTCTTTGGGTAAT TATCCTTTTCCAAAGAACATTTCCATCCCACCTGG AGTCATCCACAATAGCACATTACAGTTAGTGATG TCGACAAACTGGTTTGCCTGACAACTGTCTATCC ACAAAATCAATTGAGATCAGTTGGACTGAATCTCGA AGGGAATGGAGTGGCAACTGACGTGCCATCTGCAA CTAAAAGATGGGGCTTCAGGTCCGGTGTCCACCA AAGGTGGTCAATTATGAAGCTGGTGAATGGGCTGA AAACGTCTACAATCTTGAATCAAAAACCTGACG GGAGTGAGTGTCTACAGCAGCGCCAGACGGGATT CGGGGCTTCCCCCGGTGCCGTATGTGCACAAAGT ATCAGGAACGGGACCGTGTGCCGAGACTTTGCCT TCCACAAAGAGGGTGCTTTCTCTGTATGACCGA CTGTCTTCCACAGTTATCTACCGAGGAACGACTTTC GCTGAAGGTGTCGTTGCATTTCTGATACTGCCCA AGCTAAGAAGGACTTCTTCAGCTCACACCCCTTGA GAGAGCCGGTCAATGCAACGGAGGACCCGTCTAGT GGCTACTATTCTACCACAATTAGATATCAAGCTAC CGGTTTTGGAACCAATGAGACAGAGTATTTGTTG AGGTTGACAAATTTGACCTACGTCCAACCTGAATCA AGATTACACCACAGTTTCTGCTCCAGCTGAATGA GACAAATATACAAGTGGGAAAAGGACAAATACC ACGGGAACCAATTTGGAAGGTCAACCCCGAAAT TGATACAAACATCGGGGAGTGGGCTTCTGGGAAA CTAAAAAACCTCACTAGAAAAATTCGCAGTGAAG AGTTGTCTTTACAGCTGTATCAAACAGAGCCAAA AACATCAGTGGTCAAGTTCGGCGCAACTTCTTC CGACCCAGGGACCAACACAACACTGAAGACCAC AAAATCATGGCTTCAAAAAATCTCTGCAATGGT TCAAGTGACAGTCAAGGAAGGAAGCTGCAGTG TCGCATCTGACAACCTTGCCACAATCTCCACGAG TCCTCAACCCCCACAACCAACAGGTCCGGACA ACAGCACCCACAATACACCCGTGTATAAATTGAC ATCTCTGAGGCAACTCAAGTTGAACAACATCACCG CAGAACAGACAACGACAGCAGCCTCCGACACT CCCCCGCCACGACCGCAGCCGACCCCTAAAAGC AGAGAACACCAACACGAGCAAGGTACCGACCTC CTGGACCCCGCACCAACAAGTCCCCAAAACCA CAGCGAGACCGCTGGCAACAACAACACTCATCACC AAGATACCGGAGAAGAGAGTGCCAGCAGCGGAA GCTAGGCTTAATTACCAATACTATTGCTGGAGTCG CAGGACTGATCACAGGCGGAGGAGGCTCGAAG AGAAGCAATTGTCAATGCTCAACCCAAATGCAACC CTAATTACATTACTGGACTACTCAGGATGAAGGT GCTGCAATCGGACTGGCCTGGATACCATATTTCCG GCCAGCAGCCGAGGGAATTTACATAGAGGGGCTG ATGCACAATCAAGATGGTTTAACTCTGTGGGTGAG ACAGCTGGCCAACGAGACGACTCAAGCTCTTCAAC TGTTCTTGAGAGCCACAACCGAGCTACGCACCTTT TCAATCTCAACCGTAAGGCAATTGATTTCTTGCTG CAGCGATGGGGCGGCACATGCCACATTTGGGACC GGACTGCTGTATCGAACCACATGATTGGACCAAGA ACATAACAGACAAAATTGATCAGATTATTCATGAT TTTGTTGATAAACCTTCCGACACAGGGGACAA

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SEQ ID NO:	Description	Sequence
		TGACAATTGGTGGACAGGATGGAGACAATGGATA CCGGCAGGTATTGGAGTTACAGGCGTTATAATTGC AGTTATCGCTTTATTCTGTATATGCAAATTTGTCTT TTAG
80	Short WPRE sequence	AATCAACCTCTGGATTACAAAATTTGTGAAAGATT GACTGATATTCTTAACTATGTTGCTCCTTTTACGCT GTGTGGATATGCTGCTTTAATGCCTCTGTATCATGC TATTGCTTCCCGTACGGCTTTCGTTTTCTCCTCCTTG TATAAATCCTGGTTGCTGTCTCTTTATGAGGAGTTG TGGCCCGTTGTCCGTCAACGTGGCGTGGTGTGCTCT GTGTTTGTGACGCAACCCCACTGGCTGGGGCAT TGCCACCACCTGTCAACTCCTTCTGGGACTTTCGC TTTCCCCCTCCCGATCGCCACGGCAGAACTCATCG CCGCCTGCCTTGGCCGCTGCTGGACAGGGGCTAGG TTGCTGGGCACTGATAATTCCTGGGTGTTGTC
81	Helper Plasmid Forward Primer	TAAGCAGAATTC-ATGAATTTGCCAGGAAGAT
82	Helper Plasmid Reverse Primer	CCATACAATGAATGGACACTAGGCGGCCGCACGA AT
83	Gag, Pol, Integrase fragment	GAATTCATGAATTTGCCAGGAAGATGGAAACCAAA AATGATAGGGGAATTGGAGGTTTATCAAAGTAA GACAGTATGATCAGATACTCATAGAAATCTCGGGA CATAAAGCTATAGGTACAGTATTAGTAGGACCTAC ACCTGTCAACATAATTGGAAGAAATCTGTTGACTC AGATTGGCTGCACTTTAAATTTTCCCATTAGTCCTA TTGAGACTGTACCAGTAAATTAAGCCAGGAATG GATGGCCCAAAAGTTAAACAATGGCCATTGACAGA AGAAAAATAAAAGCATTAGTAGAAATTTGTACA GAAATGGAAAAGGAAGGAAAAATTTCAAAAATTG GGCTGAAAATCCATACAATACTCCAGTATTTGCC ATAAAGAAAAAGACAGTACTAAATGGAGAAAAAT TAGTAGATTTTCAAGAACTTAATAAGAGAACTCAA GATTTCTGGGAAGTTCAATTAGGAATACCATCC TGCAGGGTTAAACAGAAAAATCAGTAACAGTA CTGGATGTGGGCGATGCATATTTTTCAGTTCCCTTA GATAAAGACTTCAGGAAGTATACTGCATTTACCAT ACCTAGTATAAACAATGAGACACCAGGGATTAGAT ATCAGTACAATGTGCTTCCACAGGGATGGAAAGGA TCACCAGCAATATTCCAGTGTAGCATGACAAAAAT CTTAGAGCCTTTTAGAAAACAAAATCCAGACATAG TCATCTATCAATACATGGATGATTGTATGTAGGAT CTGACTTAGAAATAGGGCAGCATAGAACAAAAAT AGAGGAAC TGAGACAACATCTGTTGAGGTGGGGA TTTACCACACCAGACAAAAACATCAGAAAGAAC CTCCATTCTTTGGATGGGTTATGAACTCCATCCTG ATAAATGGACAGTACAGCCTATAGTGTGCCAGAA AAGGACAGCTGGACTGTCAATGACATACAGAAAT AGTGGGAAAATTGAATTGGGCAAGTCAGATTTATG CAGGGATTAAAGTAAGGCAATTATGTAAACTTCTT AGGGGAACCAAAGCACTAACAGAAAGTAGTACCAC TAACAGAAGAAGCAGAGCTAGAACTGGCAGAAAA CAGGGAGATTCTAAAAGAACCGGTACATGGAGTGT ATTATGACCCATCAAAAGACTTAATAGCAGAAATA CAGAAGCAGGGCAAGGCCAATGGACATATCAAA TTTATCAAGAGCCATTTAAAAATCTGAAACAGGA AAGTATGCAAGAAATGAAGGGTGCCCACTAATG ATGTGAACAATTAACAGAGGCAGTACAAAAAT AGCCACAGAAAGCATAGTAATATGGGAAAGACT CCTAAATTTAAATTACCCATACAAAGGAAACATG GGAAGCATGGTGGACAGAGTATTGGCAAGCCACCT GGATTCTGAGTGGGAGTTTGTCAATACCCCTCCCT TAGTGAAGTTATGGTACCAGTTAGAGAAAGAACCC ATAATAGGAGCAGAAACTTCTATGTAGATGGGGC AGCCAATAGGGAAACTAAATTAGGAAAGCAGGA TATGTAAC TGACAGAGGAAGACAAAAGTTGTCCC CCTAACGGACACAACAATCAGAAGACTGAGTTAC AAGCAATTCATCTAGCTTTGCAGGATTCGGGATTA GAAGTAAACATAGTGACAGACTCACAATATGCATT GGGAATCATTCAAGCACAACCAGATAAGAGTGAA TCAGAGTTAGTCAGTCAAATAATAGAGCAGTTAAT AAAAAGGAAAAAGTCTACCTGGCATGGGTACCA GCACACAAGGAATTGGAGGAAATGAACAAGTAG

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SEQ ID NO:	Description	Sequence
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84	DNA Fragment containing Rev, RRE and rabbit beta globin poly A	TCTAGAATGGCAGGAAGAAGCGGAGACAGCGACG AAGAGCTCATCAGAACAGTCAAGTCTCAAGCTT CTCATCAAAGCAACCCACCTCCCAATCCCGAGGG GACCCGACAGGCCCGAAGGAATAGAAGAAGAAGG TGGAGAGAGAGACAGAGACAGATCCATTCGATTA GTGAACGGATCCTTGGCACTTATCTGGGACGATCT GCGGAGCCTGTGCCTCTCAGCTACCAACGCTTGA GAGACTTACTCTTGATTGTAAACGAGGATTGTGGAA CTCTGGGACGACAGGGGTGGGAAGCCCTCAAATA TTGGTGAATCTCTACAATATTGGAGTCAGGAGC TAAAGAATAGAGGAGCTTTGTTCCTTGGGTTCTTG GGAGCAGCAGGAAGCACTATGGGCGCAGCGTCAA TGACGCTGACGGTACAGGCCAGACAATTATTGTCT GGTATAGTGCAGCAGCAGACAATTGTCTGAGGGC TATTGAGGCGCAACAGCATCTGTTGCAACTCACAG TCTGGGGCATCAAGCAGCTCCAGGCAAGAATCCTG GCTGTGGAAGATACCTAAGGATCAACAGCTCCT AGATCTTTTCCCTCTGCCCCAAATTTATGGGGACAT CATGAAGCCCTTGAGCATCTGACTTCTGGCTAAT AAAGGAAATTTATTTTCATTGCAATAGTGTGTTGG AATTTTTTGTGTCTCTCACTCGGAAGGACATATGGG AGGGCAAATCATTTAAACATCAGAAATGAGTATTT GGTTTAGAGTTTGGCAACATATGCCATATGTGGC TGCCATGAACAAAGGTGGCTATAAAGAGGTCA GTATATGAAACAGCCCCCTGCTGTCCATTCCTTATT CCATAGAAAAGCCTTGACTTGAGGTTAGATTTTTTT TATATTTTGTTTTGTGTTATTTTTCTTTAACATCC CTAAAATTTTCCTTACATGTTTTACTAGCCAGATTT TTCCTCCTCTCCTGACTACTCCAGTCATAGCTGTC CCTCTTCTCTTATGAAGATCCCTCGACCTGCAGCCC AAGCTTGGCGTAATCATGGTCATAGCTGTTTCCTGT GTGAAATTGTTATCCGCTCACAATTCACACAACA TACGAGCCGGAAGCATAAAGGTGTAAGCCCTGGG TGCCTAATGAGTGAGCTAACTCACATTAATTGCGT TGCCTCACTGCCCGCTTCCAGTCGGGAAACCTG TCGTGCCAGCGGATCCGATCTCAATTAGTCAGCA ACCATAGTCCCGCCCCCTAATCCGCCCATCCCGCC CCTAATCCGCCAGTTCCGCCCATTTCCGCCCCA TGGCTGACTAATTTTTTTTATTTATGCAGAGCCGA GGCCGCTCGGCCTCTGAGCTATTCCAGAAGTAGT GAGGAGGCTTTTTTGGAGGCTAGGCTTTTGCAA AAGCTAATGTTTATTGTCAGCTTATAATGGTTACA AATAAAGCAATAGCATCACAATTTACAAAATAAA GCATTTTTTCTACTGCATTCTAGTTGTGGTTGTCC AAACATCAATGTATCTTATCAGCGGCCGCCCGG GG

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SEQ ID NO:	Description	Sequence
85	DNA fragment containing the CAG enhancer/promoter/ intron sequence	ACGCGTTAGTTATTAATAGTAATCAATTACGGGGT CATTAGTTCATAGCCCATATATGGAGTTCCGCGTTA CATAACTTACGGTAATGGCCCGCTGGCTGACCG CCCAACGACCCCCGCCATTGACGTCAATAATGAC GTATGTTCCCATAGTAACGCCAATAGGGACTTTC ATTGACGTCAATGGGTGGACTATTACGGTAACT GCCCACTTGGCAGTACATCAAGTGTATCATATGCC AAGTACGCCCCCTATTGACGTCAATGACGGTAAAT GGCCCGCTGGCATTATGCCAGTACATGACCTTA TGGGACTTTCCTACTTGGCAGTACATCTACGTATTA GTCATCGCTATTACCATGGGTCGAGGTGAGCCCCA CGTTCTGCTTCACTCTCCCCATCTCCCCCCCCCTCC CACCCCCAATTTTGTATTTATTTATTTTAAATTATT TTGTGACGCGATGGGGGCGGGGGGGGGGGGGCG CGCGCCAGGCGGGGCGGGGCGGGGCGAGGGGCGG GGCGGGGCGAGGCGGAGAGGTGCGGCGGCAGCCA ATCAGAGCGGCGCGCTCCGAAAGTTTCTTTTATG GCGAGGCGGCGGCGGCGGCGGCGCTATAAAAAGC GAAGCGCGGCGGGCGGGAGTCTGCTGCGTTGCCT TCGCCCCGTGCCCCGCTCCGCGCCGCTCGCGCCG CCGCCCCGGCTCTGACTGACCGGCTACTCCAC AGGTGAGCGGCGGGACGGCCCTTCTCTCGGGC TGTAATTAGCGCTTGGTTAATGACGGCTCGTTTCT TTTCTGTGGCTGCGTGAAGCCTTAAAGGGCTCCG GGAGGGCCCTTTGTGCGGGGGGAGCGGCTCGGG GGGTGCGTGCGTGTGTGTGCGTGGGAGCGCCG CGTGCGGCGCGCTGCCCCGCGCTGTGAGCGCT GCGGGCGCGGCGGGGCTTTGTGCGCTCCGCGTG TGCGCGAGGGGAGCGCGCGCGGGGCGGTGCCCC GCGGTGCGGGGGGCTGCGAGGGGAACAAGGCT GCGTGCGGGGTGTGTGCGTGGGGGGTGAGCAGG GGGTGTGGGCGCGCGGTCGGGCTGTAAACCCCC CTGACCCCCCTCCCGAGTTGCTGAGCAGGCC GGCTTCGGGTGCGGGGCTCCGTGCGGGCGTGGCG CGGGGCTCGCCGTGCGGGCGGGGGGTGGCGGCA GGTGGGGGTGCGGGCGGGGCGGGGCGCCCTCGG GCCGGGAGGGCTCGGGGAGGGGCGCGGCGGCC CCGAGCGCGCGCGGCTGTGAGGCGCGCGGAGC CGCAGCCATTGCCTTTTATGGTAATCGTGCGAGAG GGCGCAGGACTTCTTTGTCCCAATCTGGCGGA GCCGAAATCTGGGAGGCGCGCGCACCCCCCTTA GCGGCGCGGGGCAAGCGGTGCGGCGCGGCGAGG AAGGAAATGGGCGGGGAGGGCTTCGTGCGTCCG CGCGCCCGCTCCCCCTTCTCATCTCCAGCTCGGG GCTGCCGCGAGGGGACGGCTGCCTTCGGGGGGA CGGGGCGAGGCGGGGTTCCGCTTCTGCGGTGTGAC CGCGGGGAATTC
86	DNA fragment containing VSV- G	GAATTCATGAAGTGCCTTTTGTACTTAGCCTTTTAA TTCATTGGGGTGAATTGCAAGTTACCATAGTTTTT CCACACAACCAAAAGGAACTGGAAAAATGTTT CTTCTAATTACATTATTGCCCGTCAAGCTCAGATT TAAATTGGCATAATGACTTAATAGGCACAGCCTTA CAAGTCAAAATGCCAAGAGTCACAAGGCTATTCA AGCAGACGGTTGGATGTGTATGCTTCCAATGGG TCACTACTTGTGATTTCGCTGGTATGGACCGAAGT ATATAACACATTCCATCCGATCCTTCACTCCATCTG TAGAACAATGCAAGGAAGCATTGAACAAACGAA ACAAGGAACCTGGCTGAATCCAGGCTTCCCTCCTC AAAGTTGTGGATATGCAACTGTGACGGATGCCGAA GCAGTGATTGTCCAGGTGACTCCTCACCATGTGCT GGTTGATGAATACACAGGAGTGGGTTGATTAC AGTTTCATCAACGGAATGCAAGCAATTACATATGC CCCACTGTCCATAACTCTACAACCTGGCATTCTGAC TATAAGGTCAAAGGGCTATGTGATTCTAACCTCAT TTCATGGACATCACCTTCTCTCAGAGGACGGAG AGCTATCATCCCTGGGAAAGGAGGACAGGGTTT AGAAGTAACTACTTTGCTTATGAAACTGGAGGCAA GGCCTGCAAAATGCAACTGCAAGCATGGGGAG TCAGACTCCCATCAGGTGTCTGGTTCGAGATGGCT GATAAGGATCTCTTTGCTGCAGCCAGATTCCCTGA ATGCCAGAGGGTCAAGTATCTCTGCTCCATCTC AGACCTCAGTGGATGTAAGTCTAATTGAGGACGTT GAGAGGATCTTGGATTATTCCTCTGCCAAGAAAC CTGGAGCAAAATCAGAGCGGTCTTCCAATCTCTC

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SEQ ID NO:	Description	Sequence
		CAGTGGATCTCAGCTATCTTGCTCCTAAAAACCCA GGAACCGGTCCTGCTTTACCATAATCAATGGTAC CCTAAAATACTTTGAGACCAGATACATCAGAGTCG ATATTGCTGCTCCAATCCTCTCAAGATGGTCGGA ATGATCAGTGGAACTACCACAGAAAGGGAAGTGT GGGATGACTGGGACCACATGAAGACGTGGAAATT GGACCCAATGGAGTTCTGAGGACCAGTTCAGGATA TAAGTTTCCTTTATACATGATTGGACATGGTATGTT GGACTCCGATCTTCATCTTAGCTCAAAGGCTCAGG TGTTCGAACATCCTCACATTCAAGACGCTGCTTCGC AACTTCCTGATGATGAGAGTTTATTTTTTGGTGATA CTGGGCTATCCAAAATCCAATCGAGCTTGTAGAA GGTGGTTTCAGTAGTTGGAAAAGCTCTATTGCCTCT TTTTCTTTATCATAGGGTTAATCATTGGACTATTC TTGGTTCTCCGAGTTGGTATCCATCTTGCATTAAA TTAAGCACACCAAGAAAAGACAGATTTATACAG ACATAGAGATGAGAATTC
87	Helper plasmid containing RRE and rabbit beta globin poly A	TCTAGAAGGAGCTTTGTTCTTGGGTTCTTGGGAGC AGCAGGAAGCACTATGGGCGCAGCGTCAATGACG CTGACGGTACAGGCCAGACAATTATTGTTCTGGTAT AGTGCAGCAGCAGAACAAATTTGCTGAGGGCTATTG AGGCGCAACAGCATCTGTTGCAACTCACAGTCTGG GGCATCAAGCAGCTCCAGGCAAGAAATCCTGGCTGT GGAAAGATACCTAAAGGATCAACAGCTCCTAGATC TTTTTCCTCTGCCAAAAATTTATGGGGACATCATGA AGCCCTTGTAGCATCTGACTTCTGGCTAATAAAG AAATTTATTTTCATTGCAATAGTGTGTGGAATTTT TTGTGTCTCTCACTCGGAAGGACATATGGGAGGGC AAATCATTTAAAAACATCAGAATGAGTATTTGGTTT AGAGTTTGGCAACATATGCCATATGCTGGCTGCCA TGAACAAAGGTGGCTATAAAGAGGTCATCAGTATA TGAACAGCCCCCTGCTGTCCATTCTTATTCATA GAAAAGCCTTGACTTGAGGTTAGATTTTATATAT TTTGTTTTGTGTTATTTTTTCTTTAACATCCCTAAA ATTTTCTTACATGTTTACTAGCCAGATTTTCTCTC CTCTCCTGACTACTCCAGTCATAGCTGTCTCTCTT CTCTTATGAAGATCCCTCGACCTGCAGCCCAAGCT TGGCGTAATCATGGTCATAGCTGTTTCTGTGTGAA ATTGTTATCCGCTCACAAATCCACACAACATACGA GCCGGAAGCATAAAGTGTAAGCCTGGGGTGCCTA ATGAGTGAGCTAACTCATTAAATGCGTTGCGCT CACTGCCCGCTTTCAGTCCGGAAACCTGTGCTGC CAGCGGATCCGCATCTCAATTAGTCAGCAACCATA GTCCCGCCCCCTAACTCCGCCCATCCCGCCCCTA CCGCCAGTTCCGCCCATTTCTCCGCCCATGGCTG ACTAATTTTTTTTATTTATGCAGAGGCCGAGGCCGC CTCGGCTCTGAGCTATTCCAGAAGTAGTGAGGAG GCTTTTTTGGAGGCCTAGGCTTTTGCAAAAGCTA ACTTGTTTATTGCAGCTTATAATGGTTACAAATAAA GCAATAGCATCACAAATTTCAAAATAAAGCATTT TTTTCACTGCATTCTAGTTGGTTTGTCCAAATC ATCAATGTATCTTATCACCCGGG
88	RSV promoter and HIV Rev	CAATTGCGATGTACGGGCCAGATATACGCGTATCT GAGGGGACTAGGGTGTGTTTAGGCGAAAAGCGGG GCTTCGGTTGTACGCGTTAGGAGTCCCTCAGGA TATAGTAGTTTCGCTTTTGCTAGGGAGGGGAAA TGAGTCTTATGCAATACACTTGTAGCTTGCACAA TGGTAACGATGAGTTAGCAACATGCCCTACAAAGGA GAGAAAAGCACCGTGATGCCGATTGGTGAAG TAAGGTGGTACGATCGTGCCTTATTAGGAAGGCAA CAGACAGGTCTGACATGGATTGGACGAACCACTGA ATTCCGATTGCAGAGATAATTGATTTAAGTGCCT AGCTCGATACAATAAACGCCATTTGACCATTCACC ACATTGGTGTGCACCTCCAAGCTCGAGCTCGTTTA GTGAACCGTCAGATCGCTGGAGACGCCATCCACG CTGTTTTGACCTCCATAGAAGACACCGGACCGAT CCAGCCTCCCTCGAAGCTAGCGATTAGGCATCTC CTATGGCAGGAAGAAGCGGAGACAGCGACGAAGA ACTCCTCAAGGCAGTCAGACTCATCAAGTTTCTCT ATCAAAGCAACCCACCTCCCAATCCCGAGGGGACC CGACAGGCCCGAAGGAATAGAAGAAGAGGTGGA GAGAGAGACAGAGACAGATCCATTTCGATTAGTGA ACGGATCCTTAGCACTTATCTGGACGATCTGCGG AGCCTGTGCCTCTTCAGCTACCACCGCTTGAGAGA

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SEQ ID NO:	Description	Sequence
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89	Rev/Tat shRNA Target sequence	ATGGCAGGAAGAAGCGGAG
90	Rev/Tat shRNA sequence	ATGGCAGGAAGAAGCGGAGTTCAAGAGACTCCGC TTCTTCTGCCATTTTTT
91	H1 promoter and shRT sequence	GAACGCTGACGTCATCAACCCGCTCCAAGGAATCG CGGGCCAGTGTCCTAGGCGGGAACACCCAGCGC GCGTGCGCCCTGGCAGGAAGATGGCTGTGAGGGA CAGGGGAGTGGCGCCCTGCAATATTTGCATGTCGC TATGTGTTCTGGGAAATCACCATAAACGTGAAATG TCTTTGGATTGGGAATCTTATAAGTTCTGTATGAG ACCACTTGGATCCGCGGAGACAGCGACGAAGAGC TTCAGAGAGCTCTTCGTCGCTGTCTCCGCTTTTT
92	H1 CCR5 sequence	GAACGCTGACGTCATCAACCCGCTCCAAGGAATCG CGGGCCAGTGTCCTAGGCGGGAACACCCAGCGC GCGTGCGCCCTGGCAGGAAGATGGCTGTGAGGGA CAGGGGAGTGGCGCCCTGCAATATTTGCATGTCGC TATGTGTTCTGGGAAATCACCATAAACGTGAAATG TCTTTGGATTGGGAATCTTATAAGTTCTGTATGAG ACCACTTGGATCCGCTGTCAAGTCCAATCTATGTTCA AGAGACATAGATTGGACTTGACACTTTTT
93	Primer	AGGAATTGATGGCGAGAAGG
94	Primer	CCCCAAGAAGGTCAAGTAATCA
95	Beta Actin Forward Primer	AGCGCGGCTACAGCTTCA
96	Beta Actin Reverse Primer	GGCGACGTAGCACAGCTTCP
97	AGT103 CCR5 miR30	TGTAAACTGAGCTTGCTCTA
98	AGT103-R5-1 [CCR5 miRNA target sequence]	TGTAAACTGAGCTTGCTCGC
99	AGT103-R5-2 [CCR5 miRNA target sequence]	CATAGATTGGACTTGACAC
100	CAG promoter	TAGTTATTAATAGTAATCAATTACGGGGTCATTAG TTCATAGCCCATATATGGAGTTCGCGTTACATAA CTTACGGTAAATGGCCCGCTGGCTGACCGCCAA CGACCCCGCCCATTTGACGTCAATAATGACGTATG TTCCCATAGTAACGCCAATAGGGACTTTCCATTGA CGTCAATGGGTGGACTATTTACGGTAAACTGCCCA CTTGGCAGTACATCAAGTGATCATATGCCAAGTA CGCCCCCTATTGACGTCAATGACGGTAAATGGCCC GCCTGGCATTATGCCAGTACATGACCTTATGGGA CTTTCTACTTGGCAGTACATCTACGTATTAGTCAT CGCTATTACCATGGGTCGAGGTGAGCCCCACGTTT TGCTTACTCTCCCATCTCCCCCCCCCTCCCCACCC CCAATTTTGATTATTTATTTTAAATTATTTTG CAGCGATGGGGCGGGGGGGGGGGGGCGCGCGC CAGGCGGGCGGGGCGGGGCGAGGGCGGGGCGG GGCGAGGCGGAGAGGTGCGGCGGCAGCCAATCAG AGCGGCGCGCTCCGAAAGTTTCCTTTTATGGCGAG GCGGCGGCGGCGGCCCTATAAAAAGCGAAGC GCGCGGCGGGCG
101	H1 element	GAACGCTGACGTCATCAACCCGCTCCAAGGAATCG CGGGCCAGTGTCCTAGGCGGGAACACCCAGCGC GCGTGCGCCCTGGCAGGAAGATGGCTGTGAGGGA CAGGGGAGTGGCGCCCTGCAATATTTGCATGTCGC TATGTGTTCTGGGAAATCACCATAAACGTGAAATG

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SEQ ID NO:	Description	Sequence
		TCTTTGGATTGGGAATCTTATAAGTTCTGTATGAG ACCACTT
102	3' LTR	TGGAAGGGCTAATTCACCTCCCAACGAAGATAAGAT CTGCTTTTGTGCTTGTACTGGGTCTCTGCTGGTTAGAC CAGATCTGAGCCTGGGAGCTCTCTGGCTAACTAGG GAACCCACTGCTTAAGCCTCAATAAAGCTTGCCTT GAGTGCTTCAAGTAGTGTGTGCCCGTCTGTTGTGTG ACTCTGGTAACTAGAGATCCCTCAGACCCTTTTAGT CAGTGTGGAAATCTCTAGCAGTAGTAGTTCATGT CA
103	7SK promoter	CTGCAGTATTTAGCATGCCCCACCCATCTGCAAGG CATTCTGGATAGTGTCAAAACAGCCGGAATCAAG TCCGTTTATCTCAAACCTTTAGCATTTTGGGAATAAA TGATATTGCTATGCTGGTTAAATTAGATTTAGTT AAATTTCTGCTGAAGCTCTAGTACGATAAGCAAC TTGACCTAAGTGTAAGTTGAGATTTCCTTCAGGTT TATATAGCTTGTGCGCCGCTGGCTACCTC
104	miR155 Tat	CTGGAGGCTTGCTGAAGGCTGTATGCTGTCCGCTT CTTCCTGCCATAGGGTTTGGCCACTGACTGACCCT ATGGGGAAGAAGCGGACAGGACACAAGGCCTGTT ACTAGCACTCACATGGAACAAATGGCC

While certain of the preferred embodiments of the present invention have been described and specifically exemplified above, it is not intended that the invention be limited to such

embodiments. Various modifications may be made thereto without departing from the scope and spirit of the present invention.

SEQUENCE LISTING

<160> NUMBER OF SEQ ID NOS: 104

<210> SEQ ID NO 1
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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: miR30 CCR5

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agatgggtag agcaagcaca gtttaccgct gcctactgcc tcggacttca aggggctt 118

<210> SEQ ID NO 2
<211> LENGTH: 116
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: miR21 Vif

<400> SEQUENCE: 2

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catggagttc agaagaacac atccgcactg acatttttgg atctttcatc tgacca 116

<210> SEQ ID NO 3
<211> LENGTH: 114
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: miR185 Tat

<400> SEQUENCE: 3

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ccccatggc aggcagaagc ggcaccttc ctccaatga cgcgcttctc gtcg 114

<210> SEQ ID NO 4
 <211> LENGTH: 1104
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Elongation Factor-1 alpha (EF1-alpha) promoter

<400> SEQUENCE: 4

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ctggcctctt tacgggttat ggccttcgct gtccttgaat tacttccacg cccctggctg    240
cagtacgtga ttcttgatcc cgagcttcgg gttggaagtg ggtgggagag ttcgaggcct    300
tgcgcttaag gagecccttc gcctcgtgct tgagttgagg cctggcctgg gcgctggggc    360
cgccgcgtgc gaatctgggt gcaccttcgc gcctgtctcg ctgctttcga taagtctcta    420
gccatttaaa atttttgatg acctgctgct acgctttttt tctggcaaga tagtcttgta    480
aatgcggggc aagatctgca cactggtatt tcggtttttg gggccgcggg cggcgacggg    540
gcccgtgcgt cccagcgcac atgttcggcg aggcggggcc tgcgagcgcg gccaccgaga    600
atcgagcggg ggtagtctca agctggcccg cctgctctgg tgectggcct cgcgcgcggc    660
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gagcgggcgg gtgagtcaac cacacaaagg aaaaggccct tccgtcctc agcgcgcgct    840
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tggagtacgt cgtcttttag ttggggggag gggttttatg cgatggagtt tccccacact    960
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 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: CCR5 target sequence

<400> SEQUENCE: 5

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<210> SEQ ID NO 6
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 <213> ORGANISM: Artificial Sequence
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 <223> OTHER INFORMATION: Vif target sequence

<400> SEQUENCE: 6

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<210> SEQ ID NO 7
 <211> LENGTH: 20
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence

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<220> FEATURE:
 <223> OTHER INFORMATION: Tat target sequence

<400> SEQUENCE: 7

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<210> SEQ ID NO 8
 <211> LENGTH: 126
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: TAR decoy sequence

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 aagaaa 126

<210> SEQ ID NO 9
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<400> SEQUENCE: 9

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<210> SEQ ID NO 10
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 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
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<400> SEQUENCE: 10

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 <211> LENGTH: 19
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Gag target sequence

<400> SEQUENCE: 11

gaagaaatga tgacagcat 19

<210> SEQ ID NO 12
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 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
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 <223> OTHER INFORMATION: Gag shRNA sequence

<400> SEQUENCE: 12

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<210> SEQ ID NO 13
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 <212> TYPE: DNA
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 <220> FEATURE:
 <223> OTHER INFORMATION: Pol target sequence

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<400> SEQUENCE: 13

caggagcaga tgatacag

18

<210> SEQ ID NO 14

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<220> FEATURE:

<223> OTHER INFORMATION: Pol shRNA sequence

<400> SEQUENCE: 14

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<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: CCR5 target sequence #1

<400> SEQUENCE: 15

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19

<210> SEQ ID NO 16

<211> LENGTH: 52

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: CCR5 shRNA sequence #1

<400> SEQUENCE: 16

gtgtcaagtc caatctatgt tcaagagaca tagattggac ttgacacttt tt

52

<210> SEQ ID NO 17

<211> LENGTH: 19

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: CCR5 target sequence #2

<400> SEQUENCE: 17

gagcatgact gacatctac

19

<210> SEQ ID NO 18

<211> LENGTH: 52

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: CCR5 shRNA sequence #2

<400> SEQUENCE: 18

gagcatgact gacatctact tcaagagagt agatgtcagt catgctcttt tt

52

<210> SEQ ID NO 19

<211> LENGTH: 19

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: CCR5 target sequence #3

<400> SEQUENCE: 19

gtagctctaa caggttgga

19

<210> SEQ ID NO 20

<211> LENGTH: 52

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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: CCR5 shRNA sequence #3

<400> SEQUENCE: 20

gtagctctaa caggttggat tcaagagatc caacctgtta gagctacttt tt      52

<210> SEQ ID NO 21
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: CCR5 target sequence #4

<400> SEQUENCE: 21

gttcagaaac tacctctta      19

<210> SEQ ID NO 22
<211> LENGTH: 52
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: CCR5 shRNA sequence #4

<400> SEQUENCE: 22

gttcagaaac tacctcttat tcaagagata agaggtagtt tctgaacttt tt      52

<210> SEQ ID NO 23
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: CCR5 target sequence #5

<400> SEQUENCE: 23

gagcaagctc agtttacacc      20

<210> SEQ ID NO 24
<211> LENGTH: 54
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: CCR5 shRNA sequence #5

<400> SEQUENCE: 24

gagcaagctc agtttacacc ttcaagagag gtgtaaactg agcttgcctt tttt      54

<210> SEQ ID NO 25
<211> LENGTH: 141
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: CCR5 gene, sequence 1

<400> SEQUENCE: 25

atggattatc aagtgtcaag tccaatctat gacatcaatt attatacatc ggagccctgc      60
caaaaaatca atgtgaagca aatcgcagcc cgctcctcgc ctccgctcta ctcaactggtg      120
ttcatctttg gttttgtggg c      141

<210> SEQ ID NO 26
<211> LENGTH: 633
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:

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<223> OTHER INFORMATION: CCR5 gene, sequence 2

<400> SEQUENCE: 26

```

aacatgctgg tcatacctcat cctgataaac tgcaaaaggc tgaagagcat gactgacatc      60
tacctgctca acctggccat ctctgacctg ttttctcttc ttactgtccc cttctgggct      120
cactatgctg cgcgccagtg ggactttgga aatacaatgt gtcaactctt gacagggctc      180
tattttatag gcttctcttc tggaatcttc ttcacatccc tcttgacaat cgataggtac      240
ctggctgtcg tccatgctgt gtttgcctta aaagccagga cggtcacctt tgggggtggtg      300
acaagtgtga tcacttgggt ggtggctgtg ttgctgtctc tcccaggaat catctttacc      360
agatctcaaa aagaaggtct tcattacacc tgcagctctc attttccata cagtcagtat      420
caattctgga agaatttcca gacattaaag atagtcctct tggggctggt cctgccgctg      480
cttgtcatgg tcactctgcta ctcggggaat ctaaaaactc tgcttcgggtg tcgaaatgag      540
aagaagaggc acagggtgtg gaggttcttc ttcaccatca tgattgttta ttttctcttc      600
tgggctccct acaacattgt ccttctctctg aac                                     633

```

<210> SEQ ID NO 27

<211> LENGTH: 70

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: CCR5 gene, sequence 3

<400> SEQUENCE: 27

```

accttccagg aattcttttg cctgaataat tgcagtagct ctaacaggtt ggaccaagct      60
atgcaggtga                                     70

```

<210> SEQ ID NO 28

<211> LENGTH: 140

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: CCR5 gene, sequence 4

<400> SEQUENCE: 28

```

cagagactct tgggatgacg cactgctgca tcaaccccat catctatgcc ttgtcgggg      60
agaagtccag aaactacctc ttagtcttct tccaaaagca cattgccaaa cgttcttgca      120
aatgctgttc tattttccag                                     140

```

<210> SEQ ID NO 29

<211> LENGTH: 75

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: CCR5 gene, sequence 5

<400> SEQUENCE: 29

```

caagaggctc ccgagcgagc aagctcagtt tacacccgat ccactgggga gcaggaaata      60
tctgtgggct tgtga                                     75

```

<210> SEQ ID NO 30

<211> LENGTH: 541

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: CD4 promoter sequence

<400> SEQUENCE: 30

-continued

tggtggggtt caaatgtgag cccagctgt tagccctctg caaagaaaa aaaaaaaaaa	60
aaagaacaaa gggcctagat ttccctctg agccccacce taagatgaag cctctctttt	120
caaggagtg ggggtgggt ggaggcgat cctgtcagct ttgctctctc tgtggctggc	180
agtttctcca aagggttaaca ggtgtcagct ggctgagcct aggtgaacc ctgagacatg	240
ctacctctgt cttctcatgg ctggaggcag cctttgtaag tcacagaaag tagctgaggg	300
gctctgga aaagacagcc aggggtggagg tagattggtc tttgactcct gatttaagcc	360
tgattctgct taactttttc ccttgacttt ggcattttca ctttgacatg ttccctgaga	420
gcctgggggg tggggaaccc agctccagct ggtgacgttt ggggccggcc caggcctagg	480
gtgtggagga gccttgccat cgggcttctt gtctctcttc atttaagcac gactctgcag	540
a	541

<210> SEQ ID NO 31
 <211> LENGTH: 359
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: miR30-CCR5/miR21-Vif/miR185 Tat microRNA cluster sequence

<400> SEQUENCE: 31

aggatatattg ctgttgacag tgagcgactg taaactgagc ttgctctact gtgaagccac	60
agatgggtag agcaagcaca gtttaccgct gctactgcc tcggacttca aggggcttcc	120
cgggcatctc catggctgta ccaccttgct ggggatgtg tacttctgaa cttgtgttga	180
atctcatgga gttcagaaga acacatccgc actgacattt tggatatctt catctgacca	240
gctagcgggc ctggctcgag cagggggcga gggattccgc ttcttctgc catagcgtgg	300
tcccctcccc tatggcaggc agaagcggca ccttccctcc caatgaccgc gtcttcgctc	359

<210> SEQ ID NO 32
 <211> LENGTH: 590
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Long WPRE sequence

<400> SEQUENCE: 32

aatcaacctc tgattacaaa atttgtgaaa gattgactgg tattottaac tatgttgctc	60
cttttacgct atgtggatac gctgctttaa tgcctttgta tcatgctatt gcttcccgta	120
tggttttcat tttctctcc ttgtataaat cctggttgct gtctctttat gaggagtgtt	180
ggcccggtgt caggcaacgt ggcgtggtgt gcactgtgtt tgctgacgca accccactg	240
gttggggcat tgccaccacc tgctagctcc ttccgggac ttctgcttcc cccctcccta	300
ttgccacggc ggaactcatc gccgctgcc ttgccgctg ctggacaggg gctcggtgt	360
tgggcactga caattccgtg gtgttgctgg ggaaatcatc gtcctttcct tggtgctcg	420
cctgtgttgc cacctggatt ctgcgcggga cgtccttctg ctacgtccct tcggccctca	480
atccagcgga ccttccctcc cgcggcctgc tgccggtct cgggcctctt ccggtcttc	540
gccttcgccc tcagacgagt cggatctccc ttggggcgc cteccgcct	590

<210> SEQ ID NO 33
 <211> LENGTH: 1469
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence

-continued

<220> FEATURE:

<223> OTHER INFORMATION: Elongation Factor-1 alpha (EF1-alpha)
promoter - miR30CCR5 - miR21Vif - miR185 Tat

<400> SEQUENCE: 33

```

ccggtgccta gagaaggtgg cgcggggtaa actgggaaag tgatgtcgtg tactggctcc      60
gcctttttcc cgagggtggg ggagaaccgt atataagtgc agtagtcgcc gtgaacgttc      120
tttttcgcaa cggttttgcc gccagaacac aggtaagtgc cgtgtgtggt tcccgcgggc      180
ctggcctctt tacgggttat ggcccttgcg tgccttgaat tacttccacg cccctggctg      240
cagtacgtga ttcttgatcc cgagcttcgg gttggaagtg ggtgggagag ttcgaggcct      300
tgcgttaag gagecccttc gcctcgtgct tgagttgagg cctggcctgg gcgctggggc      360
cgccgcgtgc gaatctgggt gcaccttcgc gcctgtctcg ctgctttcga taagtctcta      420
gccatttaaa atttttgatg acctgtgctg acgctttttt tctggcaaga tagtcttgta      480
aatgcgggcc aagatctgca cactggtatt tcggtttttg gggccgcggg cgcgacggg      540
gcccgtgcgt cccagcgcac atgttcggcg aggcggggcc tgcgagcgcg gccaccgaga      600
atcgacggg ggtagtctca agctggcccg cctgtctctg tgccctggcct cgcgcgcgcg      660
tgtatcgccc cgccctgggc ggcaaggctg gcccggtcgg caccagttgc gtgagcgga      720
agatggccgc tcccggccc tgctgcaggg agctcaaaat ggaggacgcg gcgctcgga      780
gagcgggccc gtgagtcaac cacacaaagg aaaagggcct tccgtcctc agcgcgcgct      840
tcattgtgact ccacggagta ccgggcgcgc tccaggcacc tcgattagtt ctcgagcttt      900
tggagtacgt cgtctttagg ttggggggag gggttttatg cgatggagtt tccccacact      960
gagtgggtgg agactgaagt taggccagct tggcacttga tgtaattctc cttggaattt     1020
gccctttttg agtttgatc ttggttcatt ctcaagctc agacagtggg tcaaagtttt     1080
tttcttccat ttcagggtgc tgatgtaca aggtatattg ctgttgacag tgagcgactg     1140
taaaactgagc ttgctctact gtgaagccac agatgggtag agcaagcaca gtttaccgct     1200
gcctactgcc tcggacttca aggggcttcc cgggcacctc catggctgta ccacctgtc     1260
gggggatgtg tacttctgaa cttgtgttga atctcatgga gttcagaaga acacatccgc     1320
actgacattt tggtatcttt catctgacca gctagcgggc ctggctcgag cagggggcgga     1380
gggattccgc ttcttctgc catagcgtgg tccctcccc tatggcaggc agaagcgga     1440
ccttccctcc caatgaccgc gtcttcgctc

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<210> SEQ ID NO 34

<211> LENGTH: 228

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Rous Sarcoma virus (RSV) promoter

<400> SEQUENCE: 34

```

gtagtcttat gcaatactct tgtagtcttg caacatggta acgatgagtt agcaacatgc      60
cttacaagga gagaaaaagc accgtgcatg ccgattgggt gaagtaaggt ggtacgatcg      120
tgccttatta ggaaggcaac agacgggtct gacatggatt ggacgaacca ctgaattgcc      180
gcattgcaga gatattgtat ttaagtgcct agctcgatac aataaacg

```

<210> SEQ ID NO 35

<211> LENGTH: 180

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

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<220> FEATURE:
<223> OTHER INFORMATION: 5' Long terminal repeat (LTR)

<400> SEQUENCE: 35

ggtctctctg gttagaccag atctgagcct gggagctctc tggctaacta gggaaccac      60
tgcttaagcc tcaataaagc ttgccttgag tgcttcaagt agtgtgtgcc cgtctgttgt      120
gtgactctgg taactagaga tccctcagac ccttttagtc agtgtggaaa atctctagca      180

<210> SEQ ID NO 36
<211> LENGTH: 41
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Psi Packaging signal

<400> SEQUENCE: 36

tacgcaaaaa attttgacta gcggaggcta gaaggagaga g                          41

<210> SEQ ID NO 37
<211> LENGTH: 233
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Rev response element (RRE)

<400> SEQUENCE: 37

aggagctttg ttccttgggt tcttgggagc agcaggaagc actatgggcg cagcctcaat      60
gacgctgacg gtacaggcca gacaattatt gtctgggata gtgcagcagc agaacaattt      120
gctgagggct attgagggcg aacagcatct gttgcaactc acagtctggg gcatcaagca      180
gctccaggca agaatcctgg ctgtggaaag atacctaaag gatcaacagc tcc          233

<210> SEQ ID NO 38
<211> LENGTH: 118
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Central polypurine tract (cPPT)

<400> SEQUENCE: 38

ttttaaaaga aaagggggga ttggggggta cagtgcaggg gaaagaatag tagacataat      60
agcaacagac atacaaacta aagaattaca aaaacaaatt acaaaattca aaatttta      118

<210> SEQ ID NO 39
<211> LENGTH: 250
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: 3' delta LTR

<400> SEQUENCE: 39

tggaagggct aattcactcc caacgaagat aagatctgct tttgcttgt actgggtctc      60
tctggttaga ccagatctga gcttgggagc tctctggcta actagggaac ccactgctta      120
agcctcaata aagcttgctt tgagtgtctc aagtagtgtg tgcccgtctg ttgtgtgact      180
ctggttaacta gagatccctc agaccctttt agtcagtgtg gaaaatctct agcagtagta      240
gttcatgtca                                250

<210> SEQ ID NO 40
<211> LENGTH: 352
<212> TYPE: DNA

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<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Helper/Rev - CMV early (CAG) enhancer -
      EnhanceTranscription

<400> SEQUENCE: 40
tagttattaa tagtaatcaa ttacggggtc attagtcat agcccatata tggagttccg      60
cgttacataa cttacggtaa atggcccggc tggctgaccg cccaacgacc cccgcccatt      120
gacgtcaata atgacgtatg ttcccatagt aacgccaata gggactttcc attgacgtca      180
atgggtggac tatttacggt aaactgccc cttggcagta catcaagtgt atcatatgcc      240
aagtacgccc cctattgacg tcaatgacgg taaatggccc gcctggcatt atgccagta      300
catgacctta tgggactttc ctacttgga gtacatctac gtattagtca tc          352

<210> SEQ ID NO 41
<211> LENGTH: 290
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Helper/Rev - Chicken beta actin (CAG)
      promoter - Transcription

<400> SEQUENCE: 41
gctattacca tgggtcgagg tgagccccac gttctgcttc actctcccca tctccccccc      60
ctcccccccc ccaattttgt atttatttat tttttaatta tttgtgcag cgatgggggc      120
gggggggggg ggggcgcgcg ccaggcgggg cgggcggggg cgaggggagg ggcggggcga      180
ggcggagagg tgcggcgcca gccaatcaga gcggcgcgct ccgaaagttt ccttttatgg      240
cgaggcgggc gcggcgggcg cctataaaa agcgaagcgc gcggcgggcg          290

<210> SEQ ID NO 42
<211> LENGTH: 960
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Helper/Rev - Chicken beta actin intron -
      Enhance gene expression

<400> SEQUENCE: 42
ggagtcgctg cgttgccttc gccccgtgcc ccgctccgcg ccgcctcgcg ccgcccggcc      60
cggctctgac tgaccgcgtt actcccacag gtgagcgggc gggacggccc ttctcctcgg      120
ggctgtaatt agcgttggg ttaatgacgg ctcgtttctt ttctgtggct gcgtgaaagg      180
cttaaagggc tccgggaggg ccctttgtgc gggggggagg ggctcggggg gtgcgtgcgt      240
gtgtgtgtgc gtggggagcg ccgcgtgcgg ccgcgctgc ccggcggtcg tgagcgctgc      300
gggcgcggcg cggggctttg tgcgctccgc gtgtgcgcga ggggagcgcg gccggggggcg      360
gtgccccgcg gtgcgggggg gctgcgaggg gaacaaaggc tgcgtgcggg gtgtgtgcgt      420
gggggggtga gcaggggggt tggggcgggc ggtcgggctg taaccccccc ctgcaccccc      480
ctccccagtg tgctgagcac ggccccggtt cgggtgcggg gctccgtgcg gggcgtggcg      540
cggggctcgc cgtgccgggc ggggggtggc ggcagggtgg ggtgccgggc ggggcggggc      600
cgctcgggc cggggagggc tcgggggagg ggcgcggcg ccccgagagc ccggcggtcg      660
tcgaggcgcg gcgagccgca gccattgcct tttatggtaa tcgtgcgaga gggcgaggg      720
acttcctttg tcccaaatct ggcggagccg aaatctggga gggcgcgcgc cccccctct      780
agcggggcg cggaagcgg tgccgcgcgc gcaggaagga aatggggcg gagggccttc      840

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gtgcgtcgcc ggcgcgcgt ccccttctcc atctccagcc tcggggctgc cgcaggggga	900
cggtctgcctt cggggggggac ggggcagggc ggggttcggc ttctggcgtg tgaccggcgg	960

<210> SEQ ID NO 43
 <211> LENGTH: 1503
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Helper/Rev - HIV Gag - Viral capsid

<400> SEQUENCE: 43
 atgggtgcga gacgctcagt attaagcggg ggagaattag atcgatggga aaaaattcgg 60
 ttaagggcag ggggaaagaa aaaatataaa ttaaacata tagtatgggc aagcagggag 120
 ctagaacgat tcgcagttaa tcttgacctg ttagaaacat cagaaggctg tagacaaata 180
 ctgggacagc tacaaccatc ccttcagaca ggatcagaag aacttagatc attatataat 240
 acagtagcaa cctctattg tgtgcatcaa aggatagaga taaaagacac caaggaagct 300
 ttagacaaga tagaggaaga gaaaacaaa agtaagaaaa aagcacagca agcagcagct 360
 gacacaggac acagcaatca ggtcagccaa aattacccta tagtgagaa catccagggg 420
 caaatggtac atcaggccat atcacctaga actttaaatg catgggtaaa agtagtagaa 480
 gagaaggctt tcagcccaga agtgataccc atgttttcag cattatcaga aggagccacc 540
 ccacaagatt taaacaccat gctaaacaca gtggggggac atcaagcagc catgcaaag 600
 ttaaaagaga ccatcaatga ggaagctgca gaatgggata gagtgcattc agtgcattgca 660
 gggcctattg caccaggcca gatgagagaa ccaaggggaa gtgacatagc aggaactact 720
 agtacccttc aggaacaaat aggatggatg acacataatc cacctatccc agtaggagaa 780
 atctataaaa gatggataat cctgggatta aataaaatag taagaatgta tagccctacc 840
 agcattctgg acataagaca aggaccaaag gaacccttta gagactatgt agaccgattc 900
 tataaaactc taagagccga gcaagcttca caagaggtaa aaaattggat gacagaaacc 960
 ttgttggtcc aaaatgcgaa ccagattgt aagactattt taaaagcatt gggaccagga 1020
 gcgacactag aagaaatgat gacagcatgt cagggagtgg ggggacccgg ccataaagca 1080
 agagttttgg ctgaagcaat gagccaagta acaaaccag ctaccataat gatacagaaa 1140
 ggcaatttta ggaaccaaag aaagactgtt aagtgtttca attgtggcaa agaagggcac 1200
 atagccaaaa attgcagggc ccctaggaaa aagggtgtt ggaaatgtgg aaaggaagga 1260
 caccaaatga aagattgtac tgagagacag gctaattttt tagggaagat ctggccttcc 1320
 cacaagggaa ggccagggaa ttttcttcag agcagaccag agccaacagc cccaccagaa 1380
 gagagcttca ggtttgggga agagacaaca actccctctc agaagcagga gccgatagac 1440
 aaggaaactgt atcctttagc ttccctcaga tcactctttg gcagcgaccc ctcgtcacaa 1500
 taa 1503

<210> SEQ ID NO 44
 <211> LENGTH: 1872
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Helper/Rev - HIV Pol - Protease and reverse transcriptase

<400> SEQUENCE: 44
 atgaatttgc caggaagatg gaaacaaaa atgatagggg gaattggagg ttttatcaaa 60

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gtaggacagt atgatcagat actcatagaa atctgcggaac ataaagctat aggtacagta	120
ttagtaggac ctacacctgt caacataatt ggaagaaatc tgttgactca gattggctgc	180
actttaaatt ttcccattag tctattgag actgtaccag taaaattaaa gccaggaatg	240
gatggcccaa aagttaaaca atggccattg acagaagaaa aaataaaagc attagtagaa	300
atttgtacag aaatggaaaa ggaaggaaaa atttcaaaaa ttgggcctga aaatccatac	360
aatactccag tatttgccat aaagaaaaaa gacagtacta aatggagaaa attagtagat	420
ttcagagaac ttaataagag aactcaagat ttctgggaag ttcaattagg aataccacat	480
cctgcagggt taaacagaa aaaatcagta acagtactgg atgtgggcga tgcataTTTT	540
tcagttccct tagataaaga cttcaggaag tatactgcat ttaccatacc tagtataaac	600
aatgagacac cagggattag atatcagtac aatgtgcttc cacagggatg gaaaggatca	660
ccagcaatat tccagtgtag catgacaaaa atcttagagc cttttagaaa acaaaatcca	720
gacatagtca tctatcaata catggatgat ttgtatgtag gatctgactt agaaataggg	780
cagcatagaa caaaaataga ggaactgaga caacatctgt tgaggtgggg atttaccaca	840
ccagacaaaa aacatcagaa agaacctcca ttcccttgga tgggttatga actccatcct	900
gataaatgga cagtacagcc tatagtgtcg ccagaaaagg acagctggac tgtcaatgac	960
atacagaaat tagtgggaaa attgaattgg gcaagtcaga tttatgcagg gattaaagta	1020
aggcaattat gtaaaacttct taggggaacc aaagcactaa cagaagtagt accactaaca	1080
gaagaagcag agctagaact ggcagaaaaac agggagattc taaaagaacc ggtacatgga	1140
gtgtattatg acccatcaaa agacttaata gcagaaatac agaagcaggg gcaaggccaa	1200
tggacatatc aaatttatca agagccattt aaaaatctga aaacaggaaa atatgcaaga	1260
atgaagggtg cccacactaa tgatgtgaaa caattaacag aggcagtaca aaaaatagcc	1320
acagaaagca tagtaatatg gggaaagact cctaaattta aattacccat acaaaaggaa	1380
acatgggaag catggtggac agagtattgg caagccacct ggattcctga gtgggagttt	1440
gtcaataccc ctcccttagt gaagttatgg taccagttag agaaagaacc cataatagga	1500
gcagaaactt tctatgtaga tggggcagcc aatagggaaa ctaaattagg aaagcagga	1560
tatgtaactg acagaggaag acaaaaagtt gtccccctaa cggacacaac aaatcagaag	1620
actgagttac aagcaattca tctagctttg caggattcgg gattagaagt aaacatagtg	1680
acagactcac aatatgcatt gggaaatcatt caagcacaac cagataagag tgaatcagag	1740
ttagtcagtc aaataataga gcagttaata aaaaaggaaa aagtctacct ggcatgggtg	1800
ccagcacaca aaggaattgg aggaaatgaa caagtagatg gggtggtcag tgetggaatc	1860
aggaaagtac ta	1872

<210> SEQ ID NO 45

<211> LENGTH: 867

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Helper Rev - HIV Integrase - Integration of viral RNA

<400> SEQUENCE: 45

tttttagatg gaatagataa ggccaagaa gaacatgaga aatatcacag taattggaga	60
gcaatggcta gtgattttta cctaccacct gtagtagcaa aagaaatagt agccagctgt	120
gataaatgtc agctaaaagg ggaagccatg catggacaag tagactgtag ccaggaata	180

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tggcagctag attgtacaca tttagaagga aaagttatct tggtagcagt tcatgtagcc	240
agtggatata tagaagcaga agtaattcca gcagagacag ggcaagaaac agcatacttc	300
ctcttaaaat tagcaggaag atggccagta aaaacagtac atacagacaa tggcagcaat	360
ttcaccagta ctacagttaa ggccgcctgt tggtagggcg ggatcaagca ggaatttggc	420
attccctaca atcccaaag tcaaggagta atagaatcta tgaataaaga attaaagaaa	480
attataggac aggttaagaga tcaggctgaa catcttaaga cagcagtaca aatggcagta	540
ttcatccaca attttaaaag aaaagggggg attggggggg acagtgcagg ggaaagaata	600
gtagacataa tagcaacaga catacaaact aaagaattac aaaaacaaat tacaaaaatt	660
caaaattttc gggtttatta cagggacagc agagatccag tttggaaagg accagcaaag	720
ctcctctgga aaggtgaagg ggcagtagta atacaagata atagtgcacat aaaagtagtg	780
ccaagaagaa aagcaaagat catcagggat tatggaaaac agatggcagg tgatgattgt	840
gtggcaagta gacaggatga ggattaa	867

<210> SEQ ID NO 46
 <211> LENGTH: 234
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Helper/Rev - HIV RRE- Binds Rev element
 <400> SEQUENCE: 46

aggagctttg ttccttgggt tcttgggagc agcaggaagc actatgggcg cagcgtaaat	60
gacgctgacg gtacaggcca gacaattatt gtctggtata gtgcagcagc agaacaattt	120
gctgagggct attgaggcgc aacagcatct gttgcaactc acagtctggg gcatcaagca	180
gctccaggca agaatcctgg ctgtggaaag atacctaaag gatcaacagc tcct	234

<210> SEQ ID NO 47
 <211> LENGTH: 351
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Helper/Rev - HIV Rev - Nuclear export and stabilize viral mRNA
 <400> SEQUENCE: 47

atggcaggaa gaagcggaga cagcgacgaa gaactcctca aggcagtcag actcatcaag	60
tttctctatc aaagcaaccc acctcccaat cccgagggga cccgacaggc ccgaaggaa	120
agaagaagaa ggtggagaga gagacagaga cagatccatt cgattagtga acggatcctt	180
agcacttata tgggacgata tgcggagcct gtgcctcttc agctaccacc gcttgagaga	240
cttactcttg attgtaacga ggattgtgga acttctggga cgcagggggg gggaaagcct	300
caaatattgg tggaatctcc tacaatattg gagtcaggag ctaaagaata g	351

<210> SEQ ID NO 48
 <211> LENGTH: 448
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Helper/Rev - Rabbit beta globin poly A - RNA stability
 <400> SEQUENCE: 48

agatcttttt ccctctgcc aaaaattatgg ggacatcatg aagccccttg agcatctgac	60
ttctggctaa taaaggaaat ttattttcat tgcaatagtg tgttggaatt ttttgtgtct	120

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ctcactcgga aggacatatg ggagggcaaa tcatttaaaa catcagaatg agtatttggg 180
ttagagtttg gcaacatatg ccatatgctg gctgccatga acaaagggtg ctataaagag 240
gtcatcagta tatgaaacag cccctgctg tccattcctt attccataga aaagccttga 300
cttgagggtta gatttttttt atattttgtt ttgtgttatt tttttcttta acatccctaa 360
aattttcctt acatgtttta ctagccagat ttttcctcct ctctgacta ctcccagtca 420
tagctgtccc tcttctctta tgaagatc 448

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<210> SEQ ID NO 49
<211> LENGTH: 352
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Helper - CMV early (CAG) enhancer -
Enhancetranscription

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<400> SEQUENCE: 49

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tagttattaa tagtaatcaa ttacgggggc attagtcat agcccatata tggagttccg 60
cgttacataa cttacggtaa atggcccgcc tggctgaccg cccaacgacc ccgcccatt 120
gacgtcaata atgacgtatg tccccatag aacgccaata gggactttcc attgacgtca 180
atgggtggac tatttacggt aaactgccca ctgggcagta catcaagtgt atcatatgcc 240
aagtacgccc cctattgacg tcaatgacgg taaatggccc gcctggcatt atgccagta 300
catgacctta tgggactttc ctacttggca gtacatctac gtattagtca tc 352

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<210> SEQ ID NO 50
<211> LENGTH: 290
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Helper - Chicken beta actin (CAG) promoter -
Transcription

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<400> SEQUENCE: 50

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gctattacca tgggtcgagg tgagcccccac gttctgcttc actctcccca tctccccccc 60
ctcccccccc ccaattttgt atttatttat tttttaatta ttttgtgcag cgatgggggc 120
gggggggggg ggggcgcgcg ccaggcgggg cggggcgggg cgaggggcgg ggcggggcga 180
ggcggagagg tgcgggcgga gccaatcaga gcggcgcgct ccgaaagtgt ccttttatgg 240
cgaggcggcg gcggcgggcg cctataaaaa agcgaagcgc gcggcgggcg 290

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<210> SEQ ID NO 51
<211> LENGTH: 960
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Helper - Chicken beta actin intron - Enhance
gene expression

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<400> SEQUENCE: 51

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ggagtgcgtg cgttgccctc gccccgtgcc ccgctccgcg ccgcctcgcg ccgcccgcgc 60
cggctctgac tgaccgcgtt actcccacag gtgagcgggc gggacggccc ttctcctccg 120
ggctgtaatt agcgttgggt ttaatgacgg ctcgtttctt ttctgtggct gcgtgaaagc 180
cttaaagggc tccgggaggg ccttttgtgc gggggggagc ggctcggggg gtgcgtgcgt 240
gtgtgtgtgc gtggggagcg ccgcgtgcgg cccgcgctgc ccggcggctg tgagcgtgc 300
gggcgcggcg cggggctttg tgcgtccgc gtgtgcgcga ggggagcgcg gccgggggcg 360

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gtgccccgcg gtgcgggggg gctgcgaggg gaacaaaggc tgcgtgcggg gtgtgtgcgt	420
gggggggtga gcaggggggtg tgggcgcggc ggtcgggctg taaccccccc ctgcaccccc	480
ctccccgagt tgctgagcac ggccccgctt cgggtgcggg gctccgtgcg gggcgtggcg	540
cggggctcgc cgtgccgggc ggggggtggc ggcaggtggg ggtgccgggc ggggcggggc	600
cgcctcgggc cggggagggc tcgggggagg ggcgcggcgg ccccgagcgc ccggcggctg	660
tcgaggcgcg gcgagccgca gccattgcct tttatggtaa tcgtgcgaga gggcgcaggg	720
acttcctttg tcccaaatct ggcgagcgcg aaatctggga ggcgccgcgc cccccctct	780
agcgggcgcg ggcgaagcgg tgcggcgcgc gcaggaagga aatgggcggg gagggccttc	840
gtgcgtcgcc gcgcgcgcgt ccccttctcc atctccagcc tcggggctgc cgcaggggga	900
cggctgcctt cggggggggc ggggcagggc ggggttcggc ttctggcggtg tgaccggcgcg	960

<210> SEQ ID NO 52

<211> LENGTH: 1503

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Helper - HIV Gag - Viral capsid

<400> SEQUENCE: 52

atgggtgcga gagegtcagt attaagcggg ggagaattag atcgatggga aaaaattcgg	60
ttaaggccag ggggaaagaa aaaatataaa ttaaaacata tagtatgggc aagcaggag	120
ctagaacgat tcgcagttaa tcctggcctg ttagaaacat cagaaggctg tagacaaata	180
ctgggacagc tacaaccatc ccttcagaca ggatcagaag aacttagatc attatataat	240
acagtagcaa ccctctattg tgtgcatcaa aggatagaga taaaagacac caaggaagct	300
ttagacaaga tagaggaaga gcaaaacaaa agtaagaaaa aagcacagca agcagcagct	360
gacacaggac acagcaatca ggtcagccaa aattacccta tagtgcagaa catccagggg	420
caaatggtac atcaggccat atcacctaga actttaaatg catgggtaaa agtagtagaa	480
gagaaggctt tcagcccaga agtgataccc atgttttcag cattatcaga aggagccacc	540
ccacaagatt taaacaccat gctaaacaca gtggggggac atcaagcagc catgcaaatg	600
ttaaagaga ccatcaatga ggaagctgca gaatgggata gagtgcattc agtgcattgca	660
gggcctattg caccaggcca gatgagagaa ccaaggggaa gtgacatagc aggaactact	720
agtacccctc aggaacaaat aggatggatg acacataatc cacctatccc agtaggagaa	780
atctataaaa gatggataat cctgggatta aataaaatag taagaatgta tagccctacc	840
agcattcttg acataagaca aggaccaaag gaacccttta gagactatgt agaccgattc	900
tataaaactc taagagccga gcaagcttca caagaggtaa aaaattggat gacagaaacc	960
ttgttggtcc aaaatgcgaa ccagattgt aagactattt taaaagcatt gggaccagga	1020
gcgacactag aagaaatgat gacagcatgt caggagtggt ggggaccggg ccataaagca	1080
agagtttttg ctgaagcaat gagccaagta acaaatccag ctaccataat gatacagaaa	1140
ggcaatttta ggaaccaaag aaagactggt aagtgtttca attgtggcaa agaagggcac	1200
atagccaaaa attgcagggc ccctaggaaa aagggtgtgt ggaaatgtgg aaaggaagga	1260
caccaaataa aagattgtac tgagagacag gctaattttt tagggaagat ctggccttcc	1320
cacaagggaa ggccagggaa ttttcttcag agcagaccag agccaacagc cccaccagaa	1380
gagagcttca ggtttgggga agagacaaca actccctctc agaagcagga gccgatagac	1440

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aaggaactgt atccttttagc ttccctcaga tcactctttg gcagcgaccc ctcgtcacia	1500
taa	1503

<210> SEQ ID NO 53
 <211> LENGTH: 1872
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Helper - HIV Pol - Protease and reverse transcriptase

<400> SEQUENCE: 53

atgaatttgc caggaagatg gaaacccaaa atgatagggg gaattggagg ttttatcaaa	60
gtaggacagt atgacagat actcatagaa atctgaggac ataaagctat aggtacagta	120
ttagtaggac ctacacctgt caacataatt ggaagaaatc tgttgactca gattggctgc	180
actttaaatt ttcccattag tcctattgag actgtaccag taaaattaaa gccaggaatg	240
gatggcccaa aagttaaaca atggccattg acagaagaaa aaataaaagc attagtagaa	300
atttgtacag aaatggaaaa ggaaggaaaa atttcaaaaa ttgggcctga aaatccatac	360
aatactccag tatttgccat aaagaaaaaa gacagtacta aatggagaaa attagtagat	420
tcagagaaac ttaataagag aactcaagat ttctgggaag ttcaattagg aataccacat	480
cctgcagggt taaacagaa aaaatcagta acagtactgg atgtgggcga tgcataatct	540
tcagttccct tagataaaga cttcaggaag tatactgcat ttaccatacc tagtataaac	600
aatgagacac cagggttagg atatcagtag aatgtgcttc cacagggatg gaaaggatca	660
ccagcaatat tccagtgtag catgacaaaa atcttagagc ctttttagaaa acaaaatcca	720
gacatagtc tctatcaata catggatgat ttgtatgtag gatctgactt agaaataggg	780
cagcatagaa caaaaataga ggaactgaga caacatctgt tgagggtggg atttaccaca	840
ccagacaaaa aacatcagaa agaacctcca ttccttttga tgggttatga actccatcct	900
gataaatgga cagtacagcc tatagtgtct ccagaaaagg acagctggac tgcataatgac	960
atacagaat tagtgggaaa attgaattgg gcaagtcaga tttatgcagg gattaaagta	1020
aggcaattat gtaaaactct taggggaacc aaagcactaa cagaagtagt accactaaca	1080
gaagaagcag agctagaact ggcagaaaa acaggagattc taaaagaacc ggtacatgga	1140
gtgtattatg acccatcaaa agacttaata gcagaaatc agaagcaggg gcaaggccaa	1200
tggacatatc aaatttatca agagccattt aaaaatctga aaacaggaaa atatgcaaga	1260
atgaagggtg cccacactaa tgatgtgaaa caattaacag aggcagtaca aaaaatagcc	1320
acagaaagca tagtaatatg gggaaagact cctaaattta aattacccat acaaaaggaa	1380
acatgggaag catggtggac agagtattgg caagccacct ggattcctga gtgggagttt	1440
gtcaataccc ctcccttagt gaagttagg taccagttag agaaagaacc cataatagga	1500
gcagaaactt tctatgtaga tggggcagcc aatagggaaa ctaaattagg aaaagcagga	1560
tatgtaactg acagaggaag acaaaaagtt gtccccctaa cggacacaac aaatcagaag	1620
actgagttac aagcaattca tctagctttg caggattcgg gattagaagt aaacatagtg	1680
acagactcac aatatgcatt gggaaatcatt caagcacacac cagataagag tgaatcagag	1740
ttagtcagtc aaataataga gcagttaata aaaaaggaaa aagtctacct ggcatgggta	1800
ccagcacaca aaggaattgg aggaaatgaa caagtagatg gggttggtcag tgctggaatc	1860
aggaaagtac ta	1872

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<210> SEQ ID NO 54
<211> LENGTH: 867
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Helper - HIV Integrase - Integration of viral
        RNA

<400> SEQUENCE: 54
tttttagatg gaatagataa ggccaagaa gaacatgaga aatatcacag taattggaga      60
gcaatggcta gtgattttaa cctaccacct gtagtagcaa aagaaatagt agccagctgt      120
gataaatgtc agctaaaagg ggaagccatg catggacaag tagactgtag cccaggaata      180
tggcagctag attgtacaca tttagaagga aaagtatatc tggtagcagt tcatgtagcc      240
agtggatata tagaagcaga agtaattcca gcagagacag ggcaagaaac agcatacttc      300
ctcttaaaat tagcaggaag atggccagta aaaacagtac atacagacaa tggcagcaat      360
ttcaccagta ctacagttaa ggccgcctgt tggtagggcg ggatcaagca ggaatttggc      420
attccctaca atcccaaaag tcaaggagta atagaatcta tgaataaaga attaaagaaa      480
attataggac aggtaagaga tcaggctgaa catcttaaga cagcagtaca aatggcagta      540
ttcatccaca attttaaaag aaaagggggg attggggggg acagtgcagg ggaagaata      600
gtagacataa tagcaacaga cataaaact aaagaattac aaaaacaaat tacaaaaatt      660
caaaattttc gggtttatta cagggacagc agagatccag tttgaaagg accagcaaag      720
ctcctctgga aagggtgaag ggccagtagta atacaagata atagtgcacat aaaagtagtg      780
ccaagaagaa aagcaaagat catcagggat tatggaaaac agatggcagg tgatgattgt      840
gtggcaagta gacaggatga ggattaa                                     867

<210> SEQ ID NO 55
<211> LENGTH: 234
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Helper - HIV RRE - Binds Rev element

<400> SEQUENCE: 55
aggagctttg ttccttgggt tcttgggagc agcaggaagc actatgggcg cagcgtcaat      60
gacgtgacg gtacaggcca gacaattatt gtctgggata gtgcagcagc agaacaattt      120
gctgagggct attgaggcgc aacagcatct gttgcaactc acagtctggg gcatcaagca      180
gctccaggca agaatcctgg ctgtggaaag atacctaaag gatcaacagc tcct          234

<210> SEQ ID NO 56
<211> LENGTH: 448
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Helper - Rabbit beta globin poly A - RNA
        stability

<400> SEQUENCE: 56
agatcttttt ccctctgcca aaaattatgg ggacatcatg aagccccttg agcatctgac      60
ttctgggctaa taaaggaaat ttattttcat tgcaatagtg tgttggaatt ttttgtgtct      120
ctcactcgga aggacatag ggagggcaaa tcatttaaaa catcagaatg agtatttggt      180
ttagagtttg gcaacatag ccatatgctg gctgccatga acaaagggtg ctataaagag      240
gtcatcagta tatgaaacag cccctgctg tccattcctt attccataga aaagccttga      300

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cttgagggtta gatttttttt atattttgtt ttgtgttatt tttttcttta acatccctaa 360
aattttcett acatgtttta ctaggcagat ttttcctcct ctcttgacta ctcccagtc 420
tagctgtccc tcttctctta tgaagatc 448

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<210> SEQ ID NO 57
<211> LENGTH: 351
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Rev - RSV promoter - Transcription

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<400> SEQUENCE: 57
atggcaggaa gaagcggaga cagcgacgaa gaactcctca aggcagtcag actcatcaag 60
tttctctatc aaagcaaccc acctcccaat cccgagggga cccgacaggc ccgaaggaat 120
agaagaagaa ggtggagaga gagacagaga cagatccatt cgattagtga acggatcctt 180
agcacttata tgggacgata tgcggagcct gtgcctcttc agctaccacc gcttgagaga 240
cttactcttg attgtaacga ggattgtgga acttctggga cgcagggggg gggaaagccct 300
caaatattgg tggaatctcc tacaatattg gagtcaggag ctaaagaata g 351

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<210> SEQ ID NO 58
<211> LENGTH: 351
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Rev - HIV Rev- Nuclear export and stabilize
viral mRNA

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<400> SEQUENCE: 58
atggcaggaa gaagcggaga cagcgacgaa gaactcctca aggcagtcag actcatcaag 60
tttctctatc aaagcaaccc acctcccaat cccgagggga cccgacaggc ccgaaggaat 120
agaagaagaa ggtggagaga gagacagaga cagatccatt cgattagtga acggatcctt 180
agcacttata tgggacgata tgcggagcct gtgcctcttc agctaccacc gcttgagaga 240
cttactcttg attgtaacga ggattgtgga acttctggga cgcagggggg gggaaagccct 300
caaatattgg tggaatctcc tacaatattg gagtcaggag ctaaagaata g 351

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<210> SEQ ID NO 59
<211> LENGTH: 450
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Rev- Rabbit beta globin poly A- RNA stability

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```

<400> SEQUENCE: 59
agatcttttt ccctctgcc aaaaattatgg ggacatcatg aagccccttg agcatctgac 60
ttctggctaa taaaggaaat ttattttcat tgcaatagtg tgttggaatt ttttgtgtct 120
ctcactcgga aggacatatg ggagggcaaa tcatttaaaa catcagaatg agtatttggt 180
ttagagtttg gcaacatatg cccatatgct ggctgccatg aacaaaggtt ggctataaag 240
aggatcatcag tatatgaaac agcccctgc tgtccattcc ttattccata gaaaagcctt 300
gacttgaggt tagatttttt ttatatattg ttttgtgtta ttttttctt taacatccct 360
aaaattttcc ttacatgttt tactagccag atttttcctc ctctcctgac tactcccagt 420
catagctgtc cctcttctct tatggagatc 450

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<210> SEQ ID NO 60
 <211> LENGTH: 577
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Envelope- CMV promoter- Transcription

<400> SEQUENCE: 60

```
acattgatta ttgactagtt attaatagta atcaattacg gggtcattag ttcatagcc 60
atatatggag ttccgcgtta cataacttac ggtaaatggc cgcctggct gaccgccc 120
cgacccccgc ccattgacgt caataatgac gtatgttccc atagtaacgc caatagggac 180
tttcattga cgtcaatggg tggagtattt acggtaaaact gccacttgg cagtacatca 240
agtgtatcat atgccaagta cgcacctat tgacgtcaat gacggtaaat ggccgcctg 300
gcattatgcc cagtacatga cttatggga ctttctact tggcagtaca tctacgtatt 360
agtcacgct attaccatgg tgatgcggtt ttggcagtac atcaatgggc gtggatagcg 420
gtttgactca cggggatttc caagtctcca cccattgac gtcaatggga gtttgtttg 480
gcacaaaaat caacgggact ttccaaaatg tcgtaacaac tccgccccat tgacgcaaat 540
gggcggtagg cgtgtacggt gggaggtcta tataagc 577
```

<210> SEQ ID NO 61
 <211> LENGTH: 573
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Envelope- Beta globin intron- Enhance gene expression

<400> SEQUENCE: 61

```
gtgagtttgg ggacccttga ttgttcttcc ttttctgcta ttgtaaaatt catgttatat 60
ggagggggca aagttttcag ggtgttgttt agaatgggaa gatgtccctt gtatcaccat 120
ggaccctcat gataattttg tttctttcac tttctactct gttgacaacc attgtctcct 180
cttattttct tttcattttc tgtaactttt tcgttaaaact ttagcttgca ttgttaacga 240
atttttaaat tcacttttgt ttatttgtca gattgtaagt actttctcta atcacttttt 300
tttcaaggca atcagggtat attatattgt acttcagcac agtttttagag aacaattgtt 360
ataattaaat gataaggtag aatattttcg catataaatt ctggctggcg tggaaatatt 420
cttattggta gaaacaacta caccctggtc atcatcctgc ctttctcttt atggttacia 480
tgatatacac tgtttgagat gaggataaaa tactctgagt ccaaaccggg cccctctgct 540
aaccatgttc atgccttctt ctctttctca cag 573
```

<210> SEQ ID NO 62
 <211> LENGTH: 1519
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Envelope- VSV-G- Glycoprotein envelope-cell entry

<400> SEQUENCE: 62

```
atgaagtgcc ttttgtactt agccttttta ttcattgggg tgaattgcaa gttcaccata 60
gtttttccac acaacaaaaa aggaaactgg aaaaatgttc cttctaatta ccattattgc 120
ccgtcaagct cagattttaa ttggcataat gacttaatag gcacagcctt acaagtcaaa 180
atgccaaga gtcacaaggc tattcaagca gacggttggg tgtgtcatgc ttccaaatgg 240
```

-continued

gtcactactt	gtgatttccg	ctggatatga	cgaagtata	taacacattc	catccgatcc	300
tctactccat	ctgtagaaca	atgcaaggaa	agcattgaac	aaacgaaaca	aggaacttgg	360
ctgaatccag	gcttccctcc	tcaaagttgt	ggatatgcaa	ctgtgacgga	tgccgaagca	420
gtgattgtcc	aggtgactcc	tcaccatgtg	ctggttgatg	aatacacagg	agaatgggtt	480
gattcacagt	tcatacaacg	aaaatgcagc	aattacatat	gccccactgt	ccataactct	540
acaacctggc	attctgaact	taaggtaaaa	gggtatgtg	attctaacct	catttccatg	600
gacatcacct	tcttctcaga	ggacggagag	ctatcatccc	tgggaaagga	gggcacaggg	660
ttcagaagta	actactttgc	ttagtaaaact	ggaggcaagg	cctgcaaaat	gcaatactgc	720
aagcattggg	gagtcagact	cccacaggt	gtctgggtcg	agatggctga	taaggatctc	780
tttgctgcag	ccagattccc	tgaatgccca	gaagggtcaa	gtatctctgc	tccatctcag	840
acctcagtg	atgtaagtct	aattcaggac	gttgagagga	tcttgatta	ttccctctgc	900
caagaaacct	ggagcaaaat	cagagcgggt	cttccaatct	ctccagtga	tctcagctat	960
cttgctccta	aaaaccagg	aaccggtcct	gctttcacca	taatcaatgg	taccctaaaa	1020
tactttgaga	ccagatacat	cagagtcgat	attgctgctc	caatcctctc	aagaatggtc	1080
ggaatgatca	gtggaactac	cacagaaagg	gaactgtggg	atgactgggc	accatatgaa	1140
gacgtggaaa	ttggacccaa	tggagtcttg	aggaccagtt	caggatataa	gtttccttta	1200
tacatgattg	gacatggtat	gttgactccc	gatcttcac	ttagctcaaa	ggctcaggtg	1260
ttcgaaacac	ctcacattca	agacgtgct	tcgcaacttc	ctgatgatga	gagtttattt	1320
tttggtgata	ctgggctatc	caaaaatcca	atcgagcttg	tagaagggtg	gttcagtagt	1380
tggaaaagct	ctattgcctc	ttttttcttt	atcataggtt	taatcattgg	actattcttg	1440
gttctccgag	ttggtatcca	tctttgcatt	aaattaaagc	acaccaagaa	aagacagatt	1500
tatacagaca	tagagatga					1519

<210> SEQ ID NO 63

<211> LENGTH: 450

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Envelope- Rabbit beta globin poly A- RNA stability

<400> SEQUENCE: 63

agatcttttt	ccctctgcc	aaaattatgg	ggacatcatg	aagccccttg	agcatctgac	60
ttctggctaa	taaaggaaat	ttattttcat	tgcaatagtg	tgttggaatt	ttttgtgtct	120
ctcactcgga	aggacatatg	ggagggcaaa	tcatttaaaa	catcagaatg	agtatttggt	180
ttagagtttg	gcaacatatg	cccatatgct	ggctgccatg	aacaaagggt	ggctataaag	240
aggtcatcag	tatatgaaac	agccccctgc	tgccattcc	ttattccata	gaaaagcctt	300
gacttgaggt	tagatttttt	ttatattttg	ttttgtgtta	ttttttctt	taacatccct	360
aaaattttcc	ttacatgttt	tactagccag	atttttcctc	ctctcctgac	tactcccagt	420
catagctgtc	cctcttctct	tatggagatc				450

<210> SEQ ID NO 64

<211> LENGTH: 1104

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Promoter- EF-1

-continued

<400> SEQUENCE: 64

```

cgggtgccta gagaaggtgg cgcggggtaa actgggaaag tgatgtcgtg tactggctcc    60
gcctttttcc cgaggggtggg ggagaaccgt atataagtgc agtagtcgcc gtgaacgttc    120
tttttcgcaa cgggttttgc gccagaaac aggtaagtgc cgtgtgtggg tcccgcgggc    180
ctggcctctt tacgggttat gcccttgcg tgccttgaat tacttccacg cccctggctg    240
cagtacgtga ttcttgatcc cgagcttcgg gttggaagtg ggtgggagag ttcgaggcct    300
tgcgcttaag gagccccctc gcctcgtgct tgagttgagg cctggcctgg gcgctggggc    360
cgccgcgtgc gaactctggt gcaccttcg gcctgtctcg ctgctttcga taagtctcta    420
gccatttaaa atttttgatg acctgctgcg acgctttttt tctggcaaga tagtcttgta    480
aatgcggggc aagatctgca cactggtatt tcggtttttg gggccgcggg cggcgacggg    540
gccccgtcgt cccagcgcac atgttcggcg aggcggggcc tgcgagcgcg gccaccgaga    600
atcggaacgg ggtagtctca agctggccgg cctgctctgg tgcctggcct cgcgcgcgcg    660
tgtatcgccc cgccctgggc ggcaaggctg gcccggtcgg caccagttgc gtgagcggaa    720
agatggccgc ttcccgccc tgctgcaggg agctcaaaat ggaggacgcg gcgctcggga    780
gagcgggcgg gtgagtcacc cacacaaagg aaaaggccct ttcgctcctc agcgcctcgt    840
tcatgtgact ccacggagta ccgggcgcg tccaggcacc tcgattagtt ctcgagcttt    900
tggagtacgt cgtctttagg ttggggggag gggttttatg cgatggagtt tccccacact    960
gagtgggtgg agactgaagt taggccagct tggcacttga tgtaattctc cttggaattt   1020
gccctttttg agtttgatc ttggttcatt ctcaagcctc agacagtggg tcaaagtttt   1080
tttcttccat ttcaggtgtc gtga                                           1104

```

<210> SEQ ID NO 65

<211> LENGTH: 511

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Promoter- PGK

<400> SEQUENCE: 65

```

gggggtgggg ttgcgccttt tccaaggcag ccctggggtt gcgcaggga cgcgctgctc    60
tgggcgtggt tccgggaaac gcagcggcgc cgacctggg tctcgacat tcttcacgtc    120
cgttcgcagc gtcacccgga tcttcgcgc tacccttggt gggcccccg cgacgcttcc    180
tgctccgccc ctaagtggg aagggtcctt gcggttcgcg gcgtgccgga cgtgacaaac    240
ggaagccgca cgtctcacta gtacctcgc agacggacag cgccaggag caatggcagc    300
gcgccgaccg cgatgggctg tggccaatag cggctgctca gcagggcgcg ccgagagcag    360
cggccgggaa ggggcggtgc gggaggcggg gtgtggggcg gtagtggtgg ccctgttcct    420
gccccgcgcg tgttcgcat tctgcaagcc tccggagcgc acgtcggcag tcggctccct    480
cgttgaccga atcaccgacc tctctcccca g                                           511

```

<210> SEQ ID NO 66

<211> LENGTH: 1162

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Promoter- UbC

<400> SEQUENCE: 66

```

gcgcggggtt ttggcgcctc ccgcgggcgc cccctcctc acggcgagcg ctgccacgtc    60

```

-continued

```

agacgaaggg cgcaggagcg ttctgatcc ttccgcccgg acgctcagga cagcggcccg 120
ctgctcataa gactcggcct tagaaccca gtatcagcag aaggacattt taggacggga 180
cttgggtgac tctagggcac tggttttctt tccagagagc ggaacaggcg aggaaaagta 240
gtcccttctc ggcgattctg cggagggatc tccgtggggc ggtgaacgcc gatgattata 300
taaggacgcg ccgggtgtgg cacagctagt tccgtcgcag ccgggatttg ggtcgcgggt 360
cttgtttgtg gatcgctgtg atcgctcactt ggtgagttgc gggctgctgg gctggccggg 420
gctttcgtgg ccgcccggcc gctcgggtgg acggaagcgt gtggagagac cgccaagggc 480
tgtagtctgg gtccgcgagc aaggttgccc tgaactgggg gttgggggga gcgcacaaaa 540
tgggcgctgt tcccagagtct tgaatggaag acgcttgtaa ggcgggctgt gaggtcgttg 600
aaacaaggtg gggggcatgg tgggcggcaa gaacccaagg tcttgaggcc ttcgctaatt 660
cgggaaagct cttattcggg tgagatgggc tggggcacca tctggggacc ctgacgtgaa 720
gtttgtcact gactggagaa ctccgggttg tctgtctggt gcggggcgcg cagttatgct 780
gtgcggttgg gcagtgcacc cgtacctttg ggagcgcgcg cctcgtcgtg tctgacgtc 840
acccgttctg ttggcttata atgcagggtg gggccacctg ccggtaggtg tgcggtaggc 900
ttttctccgt cgcaggacgc aggggttcggg cctagggtag gctctcctga atcgacaggc 960
gccggacctc tggtgagggg agggataagt gaggcgtcag tttctttggt cggttttatg 1020
tacctatctt cttaagtagc tgaagctccg gttttgaact atgcgctcgg ggttggcgag 1080
tgtgttttgt gaagtttttt aggcaccttt tgaaatgtaa tcatttgggt caatatgtaa 1140
ttttcagtgt tagactagta aa 1162

```

```

<210> SEQ ID NO 67
<211> LENGTH: 120
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Poly A- SV40

```

```

<400> SEQUENCE: 67

```

```

gtttattgca gcttataatg gttacaaata aagcaatagc atcacaaatt tcacaaataa 60
agcatttttt tcaactgcatt ctagtgtggg tttgtccaaa ctcatcaatg tatcttatca 120

```

```

<210> SEQ ID NO 68
<211> LENGTH: 227
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Poly A- bGH

```

```

<400> SEQUENCE: 68

```

```

gactgtgcct tctagttgcc agccatctgt tgtttgcccc tccccctgct cttccttgac 60
cctggaaggt gccactccca ctgtccttcc ctaataaaat gaggaaattg catcgcattg 120
tctgagtagg tgtcattcta tttcgggggg tgggggtggg caggacagca agggggagga 180
ttgggaagac aatagcaggc atgctgggga tgcgggtggc tctatgg 227

```

```

<210> SEQ ID NO 69
<211> LENGTH: 1512
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: HIV Gag- Bal

```


-continued

<400> SEQUENCE: 69

```

atgggtgcga gagcgtcagt attaagcggg ggagaattag ataggtggga aaaaattcgg    60
ttaaggccag ggggaaagaa aaaatataga ttaaacata tagtatgggc aagcagggaa    120
ctagaaagat tcgcagtcac tcctggcctg ttagaaacat cagaaggctg cagacaaata    180
ctgggacagc tacaaccatc ccttcagaca ggatcagaag aacttagatc attatataat    240
acagtagcaa cctctatttg tgtacatcaa aagatagagg taaaagacac caaggaagct    300
ttagacaaaa tagaggaaga gcaaaacaaa tgtaagaaaa aggcacagca agcagcagct    360
gacacaggaa acagcgggtca ggtcagccaa aatttcctta tagtgcagaa cctccagggg    420
caaatggtac atcaggccat atcacctaga actttaaatg catgggtaaa agtaatagaa    480
gagaaagctt tcagcccaga agtaataccc atgttttcag cattatcaga aggagccacc    540
ccacaagatt taaacaccat gctaaacaca gtggggggac atcaagcagc catgcaaatt    600
ttaaaagaac ccataatga ggaagctgca agatgggata gattgcatcc cgtgcaggca    660
gggcctgttg caccaggcca gataagagat ccaaggggaa gtgacatagc aggaactacc    720
agtacccttc aggaacaaat aggatggatg acaagtaatc cacctatccc agtaggagaa    780
atctataaaa gatggataat cctgggatta aataaaatag taaggatgta tagccctacc    840
agcatttttg acataagaca aggaccaaag gaacccttta gagactatgt agaccggttc    900
tataaaactc taagagccga gcaagcttca caggaggtaa aaaattggat gacagaaacc    960
ttgttggttc aaaatgcaaa ccagattgt aagactatct taaaagcatt gggaccagca   1020
gctacactag aagaaatgat gacagcatgt caggagtggt gaggaccagc ccataaagca   1080
agaatttttg cagaagcaat gagccaagta acaaattcag ctaccataat gatgcagaaa   1140
ggcaatttta ggaaccaaag aaagattgtt aaatgtttca attgtggcaa agaagggcac   1200
atagccagaa actgcagggc ccctaggaaa aggggctgtt ggaaatgtgg aaaggaagga   1260
caccaaatga aagactgtac tgagagacag gctaattttt tagggaaaat ctggccttcc   1320
caciaaggaa ggccagggaa ttcccttcag agcagaccag agccaacagc cccaccagcc   1380
ccaccagaag agagcttcag gtttggggaa gagacaacaa ctccctctca gaagcaggag   1440
ctgatagaca aggaactgta tcctttagct tccctcagat cactctttgg caacgacccc   1500
tcgtcacaat aa                                         1512

```

<210> SEQ ID NO 70

<211> LENGTH: 1872

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: HIV Pol- Bal

<400> SEQUENCE: 70

```

atgaatttgc caggaagatg gaaacaaaa atgatagggg gaattggagg ttttatcaaa    60
gtaagacagt atgatcagat actcatagaa atctgtggac ataaagctat aggtacagta    120
ttaataggac ctacacctgt caacataatt ggaagaaatc tgttgactca gattggttgc    180
actttaaatt ttcccattag tcctattgaa actgtaccag taaaattaaa accaggaatg    240
gatggcccaa aagttaaaaca atggccactg acagaagaaa aaataaaagc attaattggaa    300
atctgtacag aaatggaaaa ggaagggaaa atttcaaaaa ttgggcctga aaatccatac    360
aatactccag tatttgccat aaagaaaaaa gacagtacta aatggagaaa attagtagat    420
ttcagagaac ttaataagaa aactcaagac ttctgggaag tacaattagg aatacacatc    480

```

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ccgcaggggt taaaaagaa aaaatcagta acagtactgg atgtgggtga tgcataatctt 540
tcagttccct tagataaaga attcaggaag tatactgcat ttaccatacc tagtataaac 600
aatgaaacac cagggatcag atatcagtac aatgtacttc cacagggatg gaaaggatca 660
ccagcaatat ttcaaagtag catgacaaga atcttagagc cttttagaaa acaaaatcca 720
gaaatagtga tctatcaata catggatgat ttgtatgtag gatctgactt agaaataggg 780
cagcatagaa caaaaataga ggaactgaga caacatctgt tgaggtgggg atttaccaca 840
ccagacaaaa aacatcagaa agaacctcca ttcctttgga tgggttatga actccatcct 900
gataaatgga cagtacagcc tatagtgtg ccagaaaaag acagctggac tgtcaatgac 960
atacagaagt tagtgggaaa attgaattgg gcaagtcaga tttaccagg aattaaagta 1020
aagcaattat gtaggctcct taggggaacc aaggcattaa cagaagtaat accactaaca 1080
aaagaaacag agctagaact ggcagagAAC agggaaattc taaaagaacc agtacatggg 1140
gtgtattatg acccatcaaa agacttaata gcagaaatAC agaagcaggg gcaaggccaa 1200
tggacatatc aaatttatca agagccattt aaaaatctga aaacaggaaa atatgcaaga 1260
atgaggggtg cccacactaa tgatgtaaaa caattaacag aggcagtgcA aaaaataacc 1320
acagaaagca tagtaatatg gggaaagact cctaaattta aactacccat acaaaaagaa 1380
acatgggaaa catggtggac agagtattgg caagccacct ggattcctga gtgggagttt 1440
gtcaataccc ctcccttagt gaaattatgg taccagttag agaaagaacc cataatagga 1500
gcagaaacat tctatgtaga tggagcagct aaccgggaga ctaaattagg aaaagcagga 1560
tatgttacta acagaggaag acaaaaagtt gtctccctaa ctgacacaac aaatcagaag 1620
actgagttac aagcaattca tctagcttta caagattcag gattagaagt aaacatagta 1680
acagactcac aatatgcatt aggaatcatt caagcacaac cagataaaag tgaatcagag 1740
ttagtcatgc aaataataga acagttaata aaaaaggaaa aggtctacct ggcatgggta 1800
ccagcgcaaa aaggaattgg aggaaatgaa caagtagata aattagtcag tactggaatc 1860
aggaaagtac ta 1872

```

```

<210> SEQ ID NO 71
<211> LENGTH: 867
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: HIV Integrase- Bal

```

```

<400> SEQUENCE: 71

```

```

tttttagatg gaatagatat agcccaagaa gaacatgaga aatatcacag taattggaga 60
gcaatggcta gtgattttta cctgccacct gtggtagcaa aagaaatagt agccagctgt 120
gataaatgtc agctaaaagg agaagccatg catggacaag tagactgtag tccaggaata 180
tggcaactag attgtacaca tttagaagga aaaattatcc tggtagcagt tcatgtagcc 240
agtggatata tagaagcaga agttattcca gcagagacag ggcaggaaac agcatacttt 300
ctcttaaaat tagcaggaag atggccagta aaaacaatac atacagacaa tggcagcaat 360
ttcactagta ctacagtcAA ggccgcctgt tgggtggcgg ggatcaagca ggaatttggc 420
attccctaca atcccaaag tcaggagtag gtagaatcta taaataaaga attaaagaaa 480
attataggac aggttaagaga tcaggctgaa catcttaaaa cagcagtaca aatggcagta 540
ttcatccaca attttaaaag aaaagggggg attggggggg atagtgcagg ggaaagaata 600

```

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gtagacataa tagcaacaga catacaaact aaagaattac aaaaacaaat tacaaaaatt	660
caaaattttc gggttttatta cagggacagc agagatccac tttggaaagg accagcaaag	720
cttctctgga aaggtgaagg ggcagtagta atacaagata atagtgcacat aaaagtagta	780
ccaagaagaa aagcaaagat cattagggat tatggaaaac agatggcagg tgatgattgt	840
gtggcaagta gacaggatga ggattag	867

<210> SEQ ID NO 72

<211> LENGTH: 1695

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Envelope- RD114

<400> SEQUENCE: 72

atgaaactcc caacaggaat ggtcatttta tgtagcctaa taatagtctg ggcagggttt	60
gacgaccccc gcaaggctat cgcattagta caaaaacaac atggtaaacc atgcgaatgc	120
agcggagggc aggtatccga ggccccaccg aactccatcc aacaggtaac ttgccaggc	180
aagacggcct acttaatgac caacaaaaaa tggaaatgca gagtcaactcc aaaaaatctc	240
acctctagcg ggggagaact ccagaactgc cctgtgaaca ctttccagga ctcgatgcac	300
agttctgtt atactgaata ccggcaatgc agggcgaata ataagacata ctacacggcc	360
accttgctta aaatacggtc tgggagcctc aacgaggtag agatattaca aaaccccaat	420
cagctcctac agtccccttg taggggctct ataaatcagc ccgtttgctg gagtgccaca	480
gcccccatcc atatctccga tggtaggagga cccctcgata ctaagagagt gtggacagtc	540
caaaaaaggc tagaacaat tcataaggct atgcatcctg aacttcaata ccacccctta	600
gccctgcccc aagtcagaga tgacctagc cttgatgcac ggacttttga tatectgaat	660
accactttta gggtactcca gatgtccaat tttagccttg cccaagattg ttggctctgt	720
ttaaaactag gtaccctac cctctttgag ataccactc cctctttaac ctactcccta	780
gcagactccc tagcgaatgc ctctgtcag attatacctc cctcttgggt tcaaccgatg	840
cagttctcca actcgtcctg tttatcttcc cctttcatta acgatacggg acaaatagac	900
ttaggtgcag tcacctttac taactgcacc tctgtagcca atgtcagtag tcctttatgt	960
gccctaaaac ggtcagtcct cctctgtgga aataacatgg catacaccta tttaccccaa	1020
aactggacag gactttgcgt ccaagcctcc ctccctcccg acattgacat catccggggg	1080
gatgagccag tccccattcc tgccattgat cattatatac atagacctaa acgagctgta	1140
cagttcatcc ctttactagc tggactggga atcaccgcag cattcaccac cggagctaca	1200
ggcctaggtg tctccgtcac ccagtataca aaattatccc atcagttaat atctgatgtc	1260
caagtcttat ccggtacat acaagattta caagaccagg tagactcgtt agctgaagta	1320
gttctccaaa ataggagggg actggacctc ctaacggcag aacaaggagg aatttgttta	1380
gccttacaag aaaaatgctg tttttatgct aacaagtcag gaattgtgag aaacaaaata	1440
agaaccttac aagaagaatt acaaaaacgc agggaaagcc tggcatccaa ccctctctgg	1500
accgggctgc agggctttct tccgtacctc ctacctctcc tgggacccct actcaccctc	1560
ctactcatac taaccattgg gccatgcgtt ttcaatcgat tggtecaatt tgttaaagac	1620
aggatctcag tggtcaggc tctgggtttg actcagcaat atcaccagct aaaaccata	1680
gagtacgagc catga	1695

-continued

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<210> SEQ ID NO 73
<211> LENGTH: 2013
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Envelope- GALV

<400> SEQUENCE: 73
atgcttctca cctcaagccc gcaccacctt cggcaccaga tgagtctctg gagctggaaa    60
agactgatca tcctottaag ctgcgtattc ggagacggca aaacgagtct gcagaataag    120
aacccccacc agcctgtgac cctcacctgg caggtactgt cccaaactgg ggacgttgct    180
tgggacaaaa aggcagtcca gcccccttgg acttggtggc cctctcttac acctgatgta    240
tgtgccctgg cggccgtctc tgagtctctg gatatcccg gatccgatgt atcgctctct    300
aaaagagtta gacctctga ttcagactat actgccgctt ataagcaaat cacctgggga    360
gccatagggt gcagctaccc tcgggctagg accaggatgg caaattcccc cttctacgtg    420
tgtccccgag ctggccgaac ccattcagaa gctaggaggt gtggggggct agaatcccta    480
tactgtaaag aatggagttg tgagaccacg ggtaccgttt attggcaacc caagtcctca    540
tgggacctca taactgtaaa atgggaccaa aatgtgaaat gggagcaaaa atttcaaaag    600
tgtgaacaaa ccgctggtg taacccccctc aagatagact tcacagaaaa aggaaaactc    660
tcacagagatt ggataacgga aaaaacctgg gaattaaggt tctatgtata tggacaccca    720
ggcatacagt tgactatccg cttagaggtc actaacatgc cggttgtggc agtgggceca    780
gacctgttcc ttgcggaaca gggacctcct agcaagcccc tcaactctccc tctctcccca    840
cggaaaagcgc cgcccacccc tctacccccg gcggtctagt agcaaacccc tgcggtgcat    900
ggagaaactg ttaccctaaa ctctccgcct cccaccagtg gcgaccgact ctttgacctt    960
gtgcaggggg ccttccctaac ctgtgaatgt accaaccagc gggccactaa gtcttgctgg    1020
ctctgttttg gcatgagccc ccttatttat gaagggatag cctcttcagg agaggctcgt    1080
tatacctcca accatacccg atgccactgg ggggccaag gaaagcttac cctcactgag    1140
gtctccggac tcgggtcatg catagggaag gtgcctctta cccatcaaca tctttgcaac    1200
cagaccttac ccatcaattc ctctaaaaac catcagttac tgctccctc aaaccatagc    1260
tggtgggcct gcagcactgg cctcaccccc tgccctctcca cctcagtttt taatcagtct    1320
aaagacttct gtgtccaggt ccagctgata ccccgcatct attaccattc tgaagaaacc    1380
ttgttacaag cctatgacaa atcaccccc aggtttaaaa gagagcctgc ctcaactacc    1440
ctagctgtct tcctgggggt agggattgct gcaggtatag gtactggctc aaccgcccta    1500
attaagggc ccatagacct ccagcaaggc ctaaccagcc tccaaatcgc cattgacgct    1560
gacctccggg cccttcagga ctcaatcagc aagctagagg actcactgac ttccctatct    1620
gaggtagtag tccaaaatag gagaggcctt gacttactat tccttaaga aggaggcctc    1680
tgcgcgcccc taaaagaaga gtgctgtttt tatgtagacc actcagggtc agtacgagac    1740
tccatgaaaa aacttaaga aagactagat aaaagacagt tagagcgcca gaaaaacca    1800
aactgggtatg aagggtggtt caataactcc ccttggttta ctacctact atcaaccatc    1860
gctgggcccc tattgtctct cttttgttta ctactcttg ggccctgcat catcaataaa    1920
ttaatccaat tcacaaatga taggataagt gcagtcaaaa ttttagtct tagacagaaa    1980
tatcagaccc tagataacga ggaaaacctt taa                                2013

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<210> SEQ ID NO 74

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<211> LENGTH: 1530
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Envelope- FUG

<400> SEQUENCE: 74
atggttccgc aggttctttt gtttgtaact cttctgggtt ttctgtgtg ttctgggaag      60
ttccccattt acacgatacc agacgaactt ggtccctgga gccctattga catacaccat      120
ctcagctgtc caaataacct ggttgtggag gatgaaggat gtaccaacct gtccgagttc      180
tcctacatgg aactcaaagt gggatacatc tcagccatca aagtgaacgg gttcacttgc      240
acagtggttg tgacagaggc agagacctac accaactttg ttggttatgt cacaaccaca      300
ttcaagagaa agcatttccg ccccccacca gacgcatgta gagccgcgta taactggaag      360
atggccgggtg accccagata tgaagagtcc ctacacaatc cataccccga ctaccactgg      420
cttcgaactg taagaaccac caaagagtcc ctccattatc tatcccgaag tgtgacagat      480
ttggacccat atgacaaaac ccttcaactc agggctcttc ctggcggaaa gtgtcagga      540
ataacggtgt cctctaccta ctgctcaact aaccatgatt acaccatttg gatgcccgag      600
aatccgagac caaggacacc ttgtgacatt tttaccaata gcagagggaa gagagcatcc      660
aacgggaaca agacttgccg ctttgtggat gaaagaggcc tgtataagtc tctaaaagga      720
gcatgcaggc tcaagttatg tggagtcttc ggacttagac ttatggatgg aacatgggtc      780
gogatgcaaa catcagatga gaccaaatgg tgccctccag atcagttggt gaatttgac      840
gactttcgtc cagacgagat cgagcatctc gttgtggagg agttagttaa gaaaagagag      900
gaatgtctgg atgcattaga gtccatcatg accaccaagt cagtaagttt cagacgtctc      960
agtcacctga gaaaacttgt ccaggggttt ggaaaagcat ataccatatt caacaaaacc     1020
ttgatggagg ctgatgtcct ctacaagtca gtccggacct ggaatgagat catcccctca     1080
aaagggtggt tgaaagtgg aggaaggtgc catcctcatg tgaacggggt gtttttcaat     1140
gggtataatat tagggcctga cgaccatgtc ctaatcccag agatgcaatc atccctctc     1200
cagcaacata tggagtgtgt ggaatcttca gttatcccc tgatgcaccc cctggcagac     1260
ccttctacag ttttcaaaga aggtgatgag gctgaggatt ttgttgaagt tcacctcccc     1320
gatgtgtaca aacagatctc aggggttgac ctgggtctcc cgaactgggg aaagtatgta     1380
ttgatgactg caggggcoat gattggcctg gtgttgatat tttccctaata gacatgggtgc     1440
agagttggta tccatctttg cattaaatta aagcacacca agaaaagaca gatattataca     1500
gacatagaga tgaaccgact tggaaagtaa                                     1530

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<210> SEQ ID NO 75
<211> LENGTH: 1497
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Envelope- LCMV

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<400> SEQUENCE: 75
atgggtcaga ttgtgacaat gtttgaggct ctgcctcaca tcatogatga ggtgatcaac      60
attgtcatta ttgtgcttat cgtgatcacg ggtatcaagg ctgtctacaa ttttgccacc     120
tgtgggatat tcgcattgat cagtttcccta cttctggctg gcaggctcctg tggcatgtac     180
gggtcttaagg gacccgacat ttacaaagga gtttaccat ttaagtcagt ggagtttgat     240
atgtcacatc tgaacctgac catgcccacac gcatgttcag ccaacaactc ccaccattac     300

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atcagtatgg ggacttctgg actagaattg accttcacca atgattccat catcagtcac	360
aacttttgca atctgacctc tgccttcaac aaaaagacct ttgaccacac actcatgagt	420
atagtttcga gcctacacct cagtatcaga gggaactcca actataaggc agtatcctgc	480
gacttcaaca atggcataac catccaatac aacttgacat tctcagatcg aaaaagtgt	540
cagagccagt gtagaacctt cagaggtaga gtcctagata tgtttagaac tgccttcggg	600
gggaaatata tgaggagtgg ctggggctgg acaggtcag atggcaagac cacctggtgt	660
agccagacga gttaccaata cctgattata caaaatagaa cctgggaaaa ccactgcaca	720
tatgcaggtc cttttgggat gtccaggatt ctcccttccc aagagaagac taagttcttc	780
actaggagac tagcgggcac attcacctgg actttgtcag actcttcagg ggtggagaat	840
ccagtggtt attgcctgac caaatggatg attcttctg cagagcttaa gtgtttcggg	900
aacacagcag ttgcgaaatg caatgtaaat catgatgccg aattctgtga catgctgcga	960
ctaattgact acaacaaggc tgctttgagt aagttcaaag aggacgtaga atctgccttg	1020
cacttattca aaacaacagt gaattctttg atttcagatc aactactgat gaggaaccac	1080
ttgagagatc tgatgggggt gccatattgc aattactcaa agttttggta cctagaacat	1140
gcaaagaccg gcgaaactag tgctcccaag tgctggcttg tcaccaatgg ttcttactta	1200
aatgagaccc acttcagtga tcaaatcgaa caggaagccg ataacatgat tacagagatg	1260
ttgaggaagg attacataaa gaggcagggg agtaccctcc tagcattgat ggaccttctg	1320
atgttttcca catctgcata tctagtcagc atcttctgc accttgtaa aataccaaca	1380
cacaggcaca taaaaggctg ctcatgtcca aagccacacc gattaacca caaaggaatt	1440
tgtagtgtg gtgcatttaa ggtgcctggt gtaaaaaccg tctggaaaag acgctga	1497

<210> SEQ ID NO 76

<211> LENGTH: 1692

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Envelope- FPV

<400> SEQUENCE: 76

atgaacactc aaatcctggt ttctgcctt gtggcagtca tccccacaaa tgcagacaaa	60
atttgtcttg gacatcatgc tgtatcaaat ggcaccaaag taaacacact cactgagaga	120
ggagtagaag ttgtcaatgc aacggaaaca gtggagcgga caaacatccc caaaatttgc	180
tcaaaaggga aaagaaccac tgatcttggc caatgctggc tgtagggac cattaccgga	240
ccacctcaat gcgaccaatt tctagaattt tcagctgac taataatcga gagacgagaa	300
ggaaatgatg ttgtttaccc ggggaagttt gttaatgaag aggcattgcg aaaaatctc	360
agaggatcag gtgggattga caaagaaaca atgggattca catatagtgg aataaggacc	420
aacggaacaa ctagtgcatt tagaagatca gggctctcat tctatgcaga aatggagtgg	480
ctcctgtcaa atacagacaa tgctgcttcc ccacaaatga caaaatcata caaaaacaca	540
aggagagaat cagctctgat agtctgggga atccaccatt caggatcaac caccgaacag	600
accaaactat atgggagtgg aaataaactg ataacagtcg ggagttccaa atatcatcaa	660
tcttttgtgc cgagtccagg aacacgaccg cagataaatg gccagtcagg acggattgat	720
tttcattggt tgatcttggg tcccaatgat acagttactt ttagtttcaa tggggcttcc	780
atagctccaa atcgtgccag cttcttgagg ggaaagtcca tggggatcca gagcgatgtg	840

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cagggtgatg ccaattgcga aggggaatgc taccacagtg gagggactat aacaagcaga	900
ttgccttttc aaaacatcaa tagcagagca gttggcaaat gccaagata tgtaaacag	960
gaaagtttat tattggcaac tgggatgaag aacgttcccg aaccttcaa aaaaaggaaa	1020
aaaagaggcc tgtttggcgc tatagcaggg tttattgaaa atggttggga aggtctggtc	1080
gacgggtggt acggtttcag gcatcagaat gcacaaggag aaggaactgc agcagactac	1140
aaaagcacc aatcggaat tgatcagata accggaagt taaatagact cattgagaaa	1200
accaaccagc aatttgagct aatagataat gaattcactg aggtggaaaa gcagattggc	1260
aatttaatta actggaccaa agactccatc acagaagtat ggtcttcaa tgctgaactt	1320
cttgtggcaa tggaaaacca gcacactatt gatttggctg attcagagat gaacaagctg	1380
tatgagcgag tgaggaaaca attaaggga aatgctgaag aggatggcac tggttgcttt	1440
gaaatttttc ataaatgtga cgatgattgt atggctagta taaggaacaa tacttatgat	1500
cacagcaaat acagagaaga agcgatgcaa aatagaatac aaattgacct agtcaaattg	1560
agtagtggct acaaatgtgt gatactttgg tttagcttcg gggcatcatg ctttttgctt	1620
cttgccattg caatgggcct tgttttcata tgtgtgaaga acggaaacat gcggtgcact	1680
atttgtatat aa	1692

<210> SEQ ID NO 77

<211> LENGTH: 1266

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Envelope- RRV

<400> SEQUENCE: 77

agtgtaacag agcacttta tgtgtataag gctactagac catacctagc acattgcgcc	60
gattgcgggg acgggtactt ctgctatagc ccagttgcta tcgaggagat ccgagatgag	120
gcgtctgatg gcatgcttaa gatccaagtc tccgcccata taggtctgga caaggcaggc	180
acccacgccc acacgaagct ccgatatatg gctggctcatg atgttcagga atctaagaga	240
gattccttga ggggtgtacac gtccgcagcg tgctccatc atgggacgat gggacacttc	300
atcgctgcac actgtccacc aggcgactac ctcaaggttt cgttcgagga cgcagattcg	360
cacgtgaagg catgtaaggt ccaatacaag cacaatccat tgccgggtggg tagagagaag	420
ttcgtgggta gaccacactt tggcgtagag ctgccatgca cctcatacca gctgacaacg	480
gctcccaccg acgaggagat tgacatgcat acaccgccag atataccgga tcgcaccctg	540
ctatcacaga cggcggggcaa cgtcaaaata acagcaggcg gcaggactat cagggtacaac	600
tgtacctgcg gccgtgacaa cgtaggcact accagtactg acaagaccat caacacatgc	660
aagattgacc aatgccatgc tgccgtcacc agccatgaca aatggcaatt tacctctcca	720
tttgttccca ggggtgatca gacagctagg aaaggcaagg tacacgttcc gttccctctg	780
actaacgtca cctgccgagt gccgttggct cgagcgccgg atgccacctg tggttaagaag	840
gaggtgacct tgagattaca ccagatcat ccgacgctct tctcctatag gagtttagga	900
gccgaaccgc acccgtaaga ggaatgggtt gacaagttct ctgagcgcat catcccagtg	960
acggaagaag ggattgagta ccagtggggc aacaacccgc cggctctgcct gtggcgcgaa	1020
ctgacgaccg agggcaaac ccatggctgg ccacatgaaa tcattcagta ctattatgga	1080
ctataccccc ccgccactat tgccgcagta tccggggcga gtctgatggc cctcctaact	1140
ctggcgcgca catgctgcat gctggccacc gcgaggagaa agtgccctaac accgtacgcc	1200

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ctgacgccag gagecgttgg accgttgaca ctgggggtgc tttgctgcgc accgagggcg	1260
aatgca	1266

<210> SEQ ID NO 78
 <211> LENGTH: 1266
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Envelope- MLV 10A1

<400> SEQUENCE: 78

agtgtaacag agcactttaa tgtgtataag gctactagac catacctagc acattgcgcc	60
gattgcgggg acgggtactt ctgctatagc ccagttgcta tcgaggagat ccgagatgag	120
gcgtctgatg gcactgttaa gatccaagtc tccgccccaa taggtctgga caaggcaggc	180
acccacgccc acacgaagct ccgatatatg gctgggtcatg atgttcagga atctaagaga	240
gattccttga ggggtgtacac gtccgcagcg tgctccatac atgggacgat gggacacttc	300
atcgctgcac actgtccacc aggcgactac ctcaaggttt cgttcgagga cgcagattcg	360
cacgtgaagg catgtaaggc ccaatacaag cacaatccat tgccgggtggg tagagagaag	420
ttcgtgggta gaccacactt tggcgtagag ctgccatgca cctcatacca gctgacaacg	480
gctcccaccg acgaggagat tgacatgcat acaccgccag atataccgga tcgcaccctg	540
ctatcacaga cggcggggcaa cgtcaaaata acagcaggcg gcaggactat cagggtacaac	600
tgtacctgcg gccgtgacaa cgtaggcact accagtactg acaagaccat caacacatgc	660
aagattgacc aatgccatgc tgccgtcacc agccatgaca aatggcaatt tacctctcca	720
tttgttccca gggctgatca gacagctagg aaaggcaagg tacacgttcc gttccctctg	780
actaacgtca cctgccgagt gccgttggct cgagcgccgg atgccaccta tggttaagaag	840
gaggtgaccc tgagattaca ccagatcat ccgacgctct tctcctatag gagtttagga	900
gccgaaccgc acccgtaaga ggaatgggtt gacaagttct ctgagcgcat catcccagtg	960
acggaagaag ggattagta ccagtggggc aacaaccgcg cggctctgcct gtgggcgcaa	1020
ctgacgaccg agggcaaaacc ccatggctgg ccacatgaaa tcattcagta ctattatgga	1080
ctataccccc ccgcccactat tgccgcagta tccggggcga gtctgatggc cctcctaact	1140
ctggcggcca catgctgcat gctggccacc gcgaggagaa agtgcctaac accgtacgcc	1200
ctgacgccag gagecgttgg accgttgaca ctgggggtgc tttgctgcgc accgagggcg	1260
aatgca	1266

<210> SEQ ID NO 79
 <211> LENGTH: 2030
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Envelope- Ebola

<400> SEQUENCE: 79

atgggtgtta caggaatatt gcagttacct cgtgatcgat tcaagaggac atcattcttt	60
ctttgggtta ttatcctttt ccaagaaca ttttccatcc cacttgagat catccacaat	120
agcacattac aggttagtga tgcgacaaa ctggtttgcc gtgacaaact gtcacccaca	180
aatcaattga gatcagttgg actgaatctc gaagggaatg gaggggcaac tgacgtgcca	240
tctgcaacta aaagatgggg ctccaggtcc ggtgtccac caaagggtgt caattatgaa	300

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gctggtgaat gggctgaaaa ctgctacaat cttgaaatca aaaaacctga cgggagtgag	360
tgtctaccag cagcgccaga cgggattcgg ggcttcccc ggtgccgga tgtgcacaaa	420
gtatcaggaa cgggaccgtg tgccggagac ttgctctcc acaaagaggg tgctttcttc	480
ctgtatgacc gacttgcttc cacagttatc taccgaggaa cgactttcgc tgaaggtgtc	540
gttgcatctc tgatactgcc ccaagctaag aaggacttct tcagctcaca ccccttgaga	600
gagccggtca atgcaacgga ggaccgtct agtggtact attctaccac aattagatat	660
caagctaccg gttttggaac caatgagaca gagtatttgt tcgaggttga caatttgacc	720
tacgtccaac ttgaatcaag attcacacca cagtttctgc tccagctgaa tgagacaata	780
tatacaagtg ggaaggag caataccacg gaaaactaa tttggaagg caacccgaa	840
attgatacaa caatcgggga gtgggccttc tgggaaacta aaaaacctc actagaaaaa	900
ttcgagtgag agagttgtct ttccagctg tatcaaagc agccaaaaac atcagtggtc	960
agagtcgggc gcgaacttct tccgaccacg ggaccaacac aacaactgaa gaccacaaaa	1020
tcatggcttc agaaaattcc tctgcaatgg ttcaagtgc cagtcaggga agggaagctg	1080
cagtgctgca tctgacaacc ctggccacaa tctccacgag tcctcaaccc cccacaacca	1140
aaccaggtcc ggacaacagc accacaata caccgtgtg taaacttgac atctctgagg	1200
caactcaagt tgaacaacat caccgcagaa cagacaacga cagcacagcc tccgacactc	1260
cccccgccac gaccgcagcc ggaccctaa aagcagagaa caccaacacg agcaagggtg	1320
ccgacctctt ggacccgccc accacaacaa gtccccaaaa ccacagcgag accgctggca	1380
acaacaacac tcatcaccaa gataccggag aagagagtgc cagcagcggg aagctaggct	1440
taattaccaa tactattgct ggagtcgag gactgatcac aggcgggagg agagctcgaa	1500
gagaagcaat tgtcaatgct caaccctaat gcaacctaa tttacattac tggactactc	1560
aggatgaagg tgctgcaatc ggactggcct ggataccata ttctgggcca gcagccgagg	1620
gaatttcat agaggggctg atgcacaatc aagatgggtt aatctgtggg ttgagacagc	1680
tggtccacga gacgactcaa gctcttcaac tgctctgag agccacaacc gagctacgca	1740
ccttttcaat cctcaaccgt aaggcaattg atttcttctg gcagcgatgg ggcggcacat	1800
gccacatttt gggaccggac tgctgtatcg aaccacatga ttggaccaag aacataacag	1860
acaaaattga tcagattatt catgattttg ttgataaaac ccttcgggac caggggggaca	1920
atgacaattg gtggacagga tggagacaat ggataccggc aggtattgga gttacaggcg	1980
ttataattgc agttatcgct ttattctgta tatgcaaat tgtcttttag	2030

<210> SEQ ID NO 80

<211> LENGTH: 389

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Short WPRE sequence

<400> SEQUENCE: 80

aatcaacctc tggattacaa aatttgtgaa agattgactg atattcttaa ctatgttgct	60
ccttttacgc tgtgtggata tgctgcttta atgcctctgt atcatgctat tgcttccgct	120
acggctttcg ttttctcttc cttgtataaa tcctgggtgc tgtctcttta tgaggagttg	180
tggtccgttg tccgtcaacg tggcgtggtg tgctctgtgt ttgtgacgc aacccccact	240
ggctggggca ttgccaccac ctgtcaactc ctttctggga ctttcgcttt cccccctccg	300
atcgccacgg cagaactcat cgccgcctgc cttgcccgct gctggacagg ggctaggttg	360

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ctgggcactg ataattccgt ggtgtgtgc	389
<210> SEQ ID NO 81 <211> LENGTH: 31 <212> TYPE: DNA <213> ORGANISM: Artificial Sequence <220> FEATURE: <223> OTHER INFORMATION: Primer	
<400> SEQUENCE: 81	
taagcagaat tcatgaattt gccaggaaga t	31
<210> SEQ ID NO 82 <211> LENGTH: 36 <212> TYPE: DNA <213> ORGANISM: Artificial Sequence <220> FEATURE: <223> OTHER INFORMATION: Primer	
<400> SEQUENCE: 82	
ccatacaatg aatggacact aggcggccgc acgaat	36
<210> SEQ ID NO 83 <211> LENGTH: 2745 <212> TYPE: DNA <213> ORGANISM: Artificial Sequence <220> FEATURE: <223> OTHER INFORMATION: Gag, Pol, Integrase fragment	
<400> SEQUENCE: 83	
gaattcatga atttgcagg aagatggaaa ccaaaaatga tagggggaat tggaggtttt	60
atcaaagtaa gacagtatga tcagatactc atagaaatct gcggacataa agctataggt	120
acagtattag taggacctac acctgtcaac ataattggaa gaaatctgtt gactcagatt	180
ggctgcactt taaattttcc cattagtctt attgagactg taccagtaaa attaaagcca	240
ggaatggatg gcccaaaagt taaacaatgg ccattgacag aagaaaaat aaaagcatta	300
gtagaaattt gtacagaaat ggaaaaggaa ggaaaaattt caaaaattgg gcctgaaat	360
ccatacaata ctccagtatt tgccataaag aaaaaagaca gtactaaatg gagaaaaatta	420
gtagatttca gagaacttaa taagagaact caagatttct gggaagtcca attaggaata	480
ccacatcctg cagggttaaa acagaaaaaa tcagtaacag tactggatgt gggcgatgca	540
tatttttcag ttcccttaga taaagacttc aggaagtata ctgcatttac catacctagt	600
ataaacaatg agacaccagg gattagatat cagtacaatg tgcttcaca gggatggaaa	660
ggatcaccag caatattcca gtgtagcatg acaaaaatct tagagccttt tagaaaacaa	720
aatccagaca tagtcatcta tcaatacatg gatgatttgt atgtaggatc tgacttagaa	780
atagggcagc atagaacaaa aatagaggaa ctgagacaac atctgttgag gtggggattt	840
accacaccag acaaaaaaca tcagaaagaa cctccattcc tttggatggg ttatgaactc	900
catcctgata aatggacagt acagcctata gtgctgccag aaaaggacag ctggactgtc	960
aatgacatac agaaattagt gggaaaattg aattgggcaa gtcagattta tgcagggtt	1020
aaagtaaggc aattatgtaa acttcttagg ggaaccaaag cactaacaga agtagtacca	1080
ctaacagaag aagcagagct agaactggca gaaaacaggg agattctaaa agaaccggta	1140
catggagtgt attatgaccc atcaaaagac ttaatagcag aaatacagaa gcaggggcaa	1200
ggccaatgga catatcaaat ttatcaagag ccatttaaaa atctgaaaa aggaaagtat	1260

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gcaagaatga aggggtgccc cactaatgat gtgaaacaat taacagaggc agtacaaaaa	1320
ataggccacag aaagcatagt aatatgggga aagactccta aatttaaatt acccatacaa	1380
aaggaaacat ggggaagcatg gtggacagag tattggcaag ccacctggat tctgagtg	1440
gagtttgtea ataccctcc cttagtgaag ttatggtacc agttagagaa agaaccata	1500
ataggagcag aaactttcta tgtagatggg gcagccaata gggaaactaa attaggaaaa	1560
gcaggatatg taactgacag aggaagacaa aaagttgtcc ccctaacgga cacaacaaat	1620
cagaagactg agttacaagc aattcatcta gctttgcagg attcgggatt agaagtaaac	1680
atagtgcag actcacaata tgcattggga atcattcaag cacaaccaga taagagtga	1740
tcagagttag tcagtcaaat aatagagcag ttaataaaaa aggaaaaagt ctacctggca	1800
tgggtaccag cacacaaagg aattggagga aatgaacaag tagataaatt ggtcagtgct	1860
ggaatcagga aagtactatt tttagatgga atagataagg cccaagaaga acatgagaaa	1920
tatcacagta attggagagc aatggctagt gattttaacc taccacctgt agtagcaaaa	1980
gaaatagtag ccagctgtga taaatgtcag ctaaaagggg aagccatgca tggacaagta	2040
gactgtagcc caggaatatg gcagctagat tgtacacatt tagaaggaaa agttatcttg	2100
gtagcagttc atgtagccag tggatatata gaagcagaag taattccagc agagacagg	2160
caagaaacag catacttcc cttaaaatta gcaggaagat ggccagtaaa aacagtacat	2220
acagacaatg gcagcaatct caccagtact acagttaagg ccgcctgttg gtggcgggg	2280
atcaagcagg aatttggeat tccctacaat ccccaaagtc aaggagtaat agaactctatg	2340
aataaagaat taaagaaat tataggacag gtaagagatc aggctgaaca tcttaagaca	2400
gcagtacaaa tggcagtatt catccacaat tttaaaagaa aaggggggat tgggggggtac	2460
agtgccaggg aaagaatagt agacataata gcaacagaca taaaactaa agaattacaa	2520
aaacaaatta caaaaattca aaattttcgg gtttattaca gggacagcag agatccagtt	2580
tggaaaggac cagcaaagct cctctggaaa ggtgaagggg cagtagtaat acaagataat	2640
agtgacataa aagtagtgcc aagaagaaaa gcaaagatca tcagggatta tggaaaaacag	2700
atggcaggtg atgattgtgt ggcaagtaga caggatgagg attaa	2745

<210> SEQ ID NO 84

<211> LENGTH: 1586

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: DNA Fragment containing Rev, RRE and rabbit beta globin poly A

<400> SEQUENCE: 84

tctagaatgg caggaagaag cggagacagc gacgaagagc tcatcagaac agtcagactc	60
atcaagcttc tctatcaaag caaccacct cccaatcccg aggggacccg acaggcccga	120
aggaatagaa gaagaagggtg gagagagaga cagagacaga tccattcgat tagtgaacgg	180
atccttgga cttatctggg acgatctgag gagcctgtgc ctcttcagct accaccgctt	240
gagagactta ctcttgattg taacgaggat tgtggaactt ctgggacgca ggggggtggga	300
agccctcaaa tattggtgga atctcctaca atattggagt caggagctaa agaatagagg	360
agctttgttc cttgggttct tgggagcagc aggaagcact atgggcgcag cgtaaatgac	420
gtgacggta caggccagac aattattgtc tggatatagt cagcagcaga acaatttgct	480
gagggtcatt gaggcgaac agcatctgtt gcaactcaca gtctggggca tcaagcagct	540

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ccaggcaaga atcctggtctg tggaaagata cctaaaggat caacagctcc tagatctttt	600
tccctctgcc aaaaattatg gggacatcat gaagccctt gagcatctga cttctggcta	660
ataaaggaaa tttattttca ttgcaatagt gtgttggaat tttttgtgc tctcactcg	720
aaggacatat gggagggcaa atcatttaaa acatcagaat gagtatttgg tttagagttt	780
ggcaacatat gccatatgct ggctgccatg aacaagggtg gctataaaga ggcatcag	840
atatgaaaca gccccctgct gtccattcct tattccatag aaaagccttg acttgaggtt	900
agattttttt tataatttgt tttgtgttat tttttcttt aacatcccta aaattttcct	960
tacatgtttt actagccaga tttttcctcc tctcctgact actcccagtc atagctgtcc	1020
ctcttctctt atgaagatcc ctgcacctgc agcccaagct tggcgtaate atggcatag	1080
ctgtttcctg tgtgaaattg ttatccgctc acaattccac acaacatacg agccggaagc	1140
ataaagtgtg aagcctgggg tgccaatga gtgagctaac tcacattaat tgcgttgccg	1200
tcactgcccg ctttcacgct gggaaacctg tcgtgccagc ggatccgcac ctcaattagt	1260
cagcaaccat agtcccgcgc ctaactccgc ccatccgcgc ctaactccg cccagttccg	1320
cccattctcc gccccatggc tgactaatat tttttattta tgcagaggcc gagggccgct	1380
cggcctctga gctattccag aagtagtgag gaggtttttt tggaggccta ggcttttgca	1440
aaaagctaac ttgtttattg cagcttataa tggttacaaa taaagcaata gcatcacaaa	1500
tttcacaaat aaagcatttt tttcactgca ttctagtgtg ggtttgcca aactcatcaa	1560
tgtatcttat cagcggcgcc cccggg	1586

<210> SEQ ID NO 85

<211> LENGTH: 1614

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: DNA fragment containing the CAG
enhancer/promoter/intron sequence

<400> SEQUENCE: 85

acgcgttagt tattaatagt aatcaattac ggggtcatta gttcatagcc catatatgga	60
gttcgcggtt acataactta cggtaaatgg cccgcctggc tgaccgcca acgacccccg	120
cccattgacg tcaataatga cgtatgttcc catagtaacg ccaataggga ctttccattg	180
acgtcaatgg gtggactatt tacggtaaac tgcccacttg gcagtacac aagtgtatca	240
tatgccaaagt acgcccccta ttgacgtcaa tgacggtaaa tggcccgccg ggcattatgc	300
ccagtacatg accttatggg actttcctac ttggcagtac atctacgtat tagtcatcgc	360
tattaccatg ggtcgagggt agccccacgt tctgcttcac tctcccccac tccccccct	420
ccccaccccc aattttgtat ttatttatat tttaattatt ttgtgcagcg atggggcgcg	480
gggggggggg ggcgcgcgcgc aggcggggcg gggcggggcg aggggcgggg cgggcgagg	540
cggagagggt cggcggcgcgc caatcagagc ggcgcgcctc gaaagtttcc ttttatggcg	600
aggcggcggc ggcggcggcgc ctataaaaag cgaagcgcgc ggcggcgggg agtcgctgcg	660
ttgccttcgc cccgtgcccc gctccgcgcgc gcctcgcgcgc gcccgccccg gctctgactg	720
accgcgttac tcccacaggt gagcggggcg gacggccctt ctctccggg ctgtaattag	780
cgtttggttt aatgacggct cgtttctttt ctgtggctgc gtgaaagcct taaagggtc	840
cgggagggcc ctttgtgcgg gggggagcgg ctcggggggg gcgtgcgtgt gtgtgtgcgt	900
ggggagcgcc gcgtgcggcc cgcgctgccc ggcgctgtg agcgctgcgg gcgcggcgcg	960

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gggctttgtg cgctcccggt gtgcgcgagg ggagcgcggc cgggggcggg gccccgcggg 1020
gcgggggggc tgcgagggga acaaaggctg cgtgcggggg gtgtgcgtgg gggggtgagc 1080
agggggtgtg ggcgcggcgg tcgggctgta accccccctt gcacccccct ccccgagttg 1140
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tgccgggcgg ggggtggcgg cagggtggggg tgccgggcgg ggcggggcgg cctcgggcgg 1260
gggagggctc ggggaggggg cgcggcggcc ccggagcgcc ggcggctgtc gaggcgcggc 1320
gagccgcagc cattgccttt tatggtaatc gtgcgagagg gcgcagggac ttcctttgtc 1380
ccaaatctgg cggagccgaa atctgggagg cggcgccgca cccctctag cgggcgcggg 1440
cgaagcggtg cggcgccggc aggaaggaaa tgggcgggga gggccttcgt gcgtcgccgc 1500
gccgcgctcc cttcttccat ctccagctc ggggctgccg cagggggacg gctgccttcg 1560
ggggggacgg ggcagggcgg ggttcggctt ctggcggtgt accggcggga attc 1614

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<210> SEQ ID NO 86

<211> LENGTH: 1531

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: DNA fragment containing VSV-G

<400> SEQUENCE: 86

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gaattcatga agtgcccttt gtacttagcc tttttattca ttggggtgaa ttgcaagttc 60
accatagttt ttccacacaa ccaaaaagga aactggaaaa atgttccttc taattaccat 120
tattgcccggt caagctcaga tttaaattgg cataatgact taataggcac agccttacia 180
gtcaaaatgc ccaagagtca caaggctatt caagcagacg gttggatgtg tcatgcttcc 240
aaatgggtca ctacttgtga ttcccgctgg tatggaccga agtatataac acattccatc 300
cgatccttca ctccatctgt agaacaatgc aaggaaagca ttgaacaaac gaaacaagga 360
acttggtgta atccaggctt cctcctcaa agttgtggat atgcaactgt gacggatgcc 420
gaagcagtga ttgtccaggt gactcctcac catgtgctgg ttgatgaata cacaggagaa 480
tgggttgatt cacagtteat caacggaaaa tgcagcaatt acatatgcc cactgtccat 540
aactctacia cctggcattc tgactataag gtcaaaaggg tatgtgatc taacctcatt 600
tccatggaca tcacctctt ctccagaggc ggagagctat catccctggg aaaggagggc 660
acagggttca gaagtaacta ctttgcttat gaaactggag gcaaggcctg caaatgcaa 720
tactgcaagc attggggagt cagactccca tcagggtgtc ggttcgagat ggctgataag 780
gatctctttg ctgcagccag attcctgaa tgcccagaag ggtcaagtat ctctgctcca 840
tctcagacct cagtggatgt aagtctaatt caggacgttg agaggatctt ggattattcc 900
ctctgccaa gaaacctggg caaaatcaga gcgggtcttc caatctctcc agtggatctc 960
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atggtcggaa tgatcagtgg aactaccaca gaaagggaac tgtgggatga ctgggcacca 1140
tatgaagacg tggaaattgg acccaatgga gttctgagga ccagttcagg atataagttt 1200
cctttataca tgattggaca tggatgtgtg gactccgac ttcattcttag ctcaaaggct 1260
cagggtgttc aacatctca cattcaagac gctgcttcgc aacttcctga tgatgagagt 1320
ttattttttg gtgatactgg gctatccaaa aatccaatcg agctttaga aggttggttc 1380
agtagttgga aaagctctat tgcctctttt ttctttatca taggggtaat cattggacta 1440

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ttcttggttc tccgagttgg tatccatctt tgcattaaat taaagcacac caagaaaaga 1500
cagatttata cagacataga gatgagaatt c 1531

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<210> SEQ ID NO 87
<211> LENGTH: 1227
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Helper plasmid containing RRE and rabbit beta
        globin poly A

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<400> SEQUENCE: 87

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tctagaagga gctttgttcc ttgggttctt gggagcagca ggaagcacta tgggcgcagc 60
gtcaatgaag ctgacggtac aggccagaca attattgtct ggtatagtgc agcagcagaa 120
caatttgctg agggctattg aggcgcaaca gcattctgtt caactcacag tctggggcat 180
caagcagctc caggcaagaa tcttggtgtt ggaaagatac ctaaaggatc aacagctcct 240
agatcttttt ccctctgcca aaaattatgg ggacatcatg aagccccttg agcatctgac 300
ttctggctaa taaaggaaat ttattttcat tgcaatagtg tgttggaatt ttttgtgtct 360
ctcactcgga aggacatagc ggagggcaaa tcatttaaaa catcagaatg agtatttggt 420
ttagagtttg gcaacatagc ccatatgctg gctgccatga acaaaggtgg ctataaagag 480
gtcatcagta tatgaaacag cccctgctg tccattcctt attccataga aaagccttga 540
cttgaggtta gatttttttt atattttgtt ttgtgttatt tttttcttta acatccctaa 600
aattttcctt acatgtttta ctagccagat ttttcctcct ctctgacta ctcccagtca 660
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tggtcatagc tgtttctgtg gtgaaattgt tatccgctca caattccaca caacatacga 780
gccggaagca taaagtgtaa agcctggggg gcctaatagag tgagctaact cacattaatt 840
gcgttgcgct cactgccgcg ttccagtcg ggaaacctgt cgtgccagcg gatccgcatc 900
tcaattagtc agcaaccata gtcccgcgcc taactccgcc catcccgcgc ctaactccgc 960
ccagttccgc ccattctcgc ccccatggct gactaatttt tttattttat gcagaggccg 1020
aggccgcctc ggctctgtag ctattccaga agtagtgagg aggccttttt ggaggcctag 1080
gcttttgcaa aaagctaact tgtttattgc agcttataat gggtacaaat aaagcaatag 1140
catcacaat ttcacaaata aagcattttt ttcactgcat tctagttgtg gtttgtccaa 1200
actcatcaat gtatcttctc acccgggg 1227

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<210> SEQ ID NO 88
<211> LENGTH: 884
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: RSV promoter and HIV Rev

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<400> SEQUENCE: 88

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caattgcgat gtacgggcca gatatacgcg tatctgaggg gactagggtg tgtttaggcg 60
aaaagcgggg cttegggtgt acgcgggttag gagtcccctc aggatatagt agtttcgctt 120
tgtcataggg agggggaaat gtagtcttat gcaatacact tgtagtcttg caacatggta 180
acgatgagtt agcaacatgc cttacaagga gagaaaaagc accgtgcatg ccgattgggtg 240
gaagtaaggt ggtacgatcg tgccttatta ggaaggcaac agacaggtct gacatggatt 300
ggacgaacca ctgaattccg cattgcagag ataattgtat ttaagtgcct agctcgatac 360

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aataaacgcc atttgaccat tcaccacatt ggtgtgcacc tccaagctcg agctcgttta	420
gtgaaccgtc agatcgccctg gagacgccat ccacgtgtt ttgacctcca tagaagacac	480
cgggaccgat ccagcctccc ctccaagcta gcgattaggc atctcctatg gcaggaagaa	540
gcggagacag cgacgaagaa ctctcaagg cagtcagact catcaagttt ctctatcaaa	600
gcaaccaccc tcccaatccc gaggggaccc gacaggcccg aaggaataga agaagaaggt	660
ggagagagag acagagacag atccattcga ttagtgaacg gatccttagc acttatctgg	720
gacgatctgc ggagcctgtg cctcttcagc taccaccgct tgagagactt actcttgatt	780
gtaacgagga ttgtggaact tctgggacgc aggggggtggg aagccctcaa atattggtgg	840
aatctcctac aatattggag tcaggagcta aagaatagtc taga	884

<210> SEQ ID NO 89
 <211> LENGTH: 19
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Target sequence

<400> SEQUENCE: 89

atggcaggaa gaagcggag	19
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<210> SEQ ID NO 90
 <211> LENGTH: 52
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: shRNA sequence

<400> SEQUENCE: 90

atggcaggaa gaagcggagt tcaagagact ccgcttcttc ctgccatttt tt	52
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<210> SEQ ID NO 91
 <211> LENGTH: 279
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: H1 promoter and shRT sequence

<400> SEQUENCE: 91

gaacgctgac gtcatcaacc cgctccaagg aatcgcgggc ccagtgtcac taggcgggaa	60
caccacgcgc gcgtgcgccc tggcaggaag atggctgtga gggacagggg agtggcgccc	120
tgcaatatatt gcatgtcgct atgtgttctg ggaaatcacc ataacgtga aatgtctttg	180
gatttgggaa tcttataagt tctgtatgag accacttgga tccgcggaga cagcgacgaa	240
gagcttcaag agagctcttc gtcgctgtct ccgcttttt	279

<210> SEQ ID NO 92
 <211> LENGTH: 275
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: H1 CCR5 sequence

<400> SEQUENCE: 92

gaacgctgac gtcatcaacc cgctccaagg aatcgcgggc ccagtgtcac taggcgggaa	60
caccacgcgc gcgtgcgccc tggcaggaag atggctgtga gggacagggg agtggcgccc	120
tgcaatatatt gcatgtcgct atgtgttctg ggaaatcacc ataacgtga aatgtctttg	180

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gatttgggaa tcttataagt tctgtatgag accacttgga tccgtgtcaa gtccaatcta 240

tgttcaagag acatagattg gacttgacac ttttt 275

<210> SEQ ID NO 93
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 93

aggaattgat ggcgagaagg 20

<210> SEQ ID NO 94
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 94

ccccaagaa ggtcaaggta atca 24

<210> SEQ ID NO 95
<211> LENGTH: 18
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 95

agcgcggtca cagcttca 18

<210> SEQ ID NO 96
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (20)..(20)
<223> OTHER INFORMATION: n = p

<400> SEQUENCE: 96

ggcgacgtag cacagcttcn 20

<210> SEQ ID NO 97
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: AGT103 CCR5 mir30

<400> SEQUENCE: 97

tgtaaaactga gcttgctcta 20

<210> SEQ ID NO 98
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: AGT103-R5-1

<400> SEQUENCE: 98

tgtaaaactga gcttgctcgc 20

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<210> SEQ ID NO 99
 <211> LENGTH: 19
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: AGT103-R5-2

<400> SEQUENCE: 99

catagattgg acttgacac 19

<210> SEQ ID NO 100
 <211> LENGTH: 642
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: CAG promoter

<400> SEQUENCE: 100

tagttattaa tagtaatcaa ttacgggggc attagttcat agcccatata tggagttccg 60
 cgttacataa cttacggtaa atggcccgcc tggtgacgc cccaacgacc ccgcgccatt 120
 gacgtcaata atgacgtatg ttcccatagt aacgccaata gggactttcc attgacgtca 180
 atgggtggac tatttacggt aaactgccca ctggcagta catcaagtgt atcatatgcc 240
 aagtacgccc cctattgacg tcaatgacgg taaatggccc gcctggcatt atgccagta 300
 catgacctta tgggactttc ctacttgga gtacatctac gtattagtca tcgctattac 360
 catgggtcga ggtgagcccc acgtttctgt tcaactctcc catctcccc ccctccccac 420
 ccccaatttt gtattttatt attttttaat tattttgtgc agcgatgggg gcgggggggg 480
 gggggggcgc cgccaggcgg ggcggggcgg ggcgaggggc ggggcggggc gaggcggaga 540
 ggtgcggcgg cagccaatca gagcggcgcg ctccgaaagt ttccttttat ggcgagggcg 600
 cggcgggcgg gccctataa aaagcgaagc gcgcggcggg cg 642

<210> SEQ ID NO 101
 <211> LENGTH: 217
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: H1 element

<400> SEQUENCE: 101

gaacgctgac gtcatcaacc cgtccaagg aatcgcgggc ccagtgtcac taggcgggaa 60
 caccagcgc gcgtgcgcc tggcaggaag atggctgtga gggacagggg agtggcgccc 120
 tgcaatatat gcatgtcgct atgtgttctg ggaaatcacc ataaacgtga aatgtctttg 180
 gatttgggaa tcttataagt tctgtatgag accactt 217

<210> SEQ ID NO 102
 <211> LENGTH: 250
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: 3' LTR

<400> SEQUENCE: 102

tggaagggtc aattcactcc caacgaagat aagatctgct ttttgcctgt actgggtctc 60
 tctggttaga ccagatctga gcctgggagc tctctggcta actagggaac ccactgctta 120
 agcctcaata aagcttgcc tgaagtcttc aagtagtgtg tgcccgctcg ttgtgtgact 180

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ctggtaacta gagatccctc agaccctttt agtcagtgtg gaaaatctct agcagtagta    240
gttcatgtca                                                                250
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<210> SEQ ID NO 103
<211> LENGTH: 243
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: 7SK promoter
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<400> SEQUENCE: 103

ctgcagtatt tagcatgccc caccatctg caaggcattc tggatagtgt caaaacagcc    60
ggaaatcaag tccgtttatc tcaaaattta gcattttggg aataaatgat atttgctatg    120
ctggttaaat tagatttttag ttaaatttcc tgctgaagct ctagtacgat aagcaacttg    180
acctaagtgt aaagttgaga tttccttcag gtttatatag cttgtgcgcc gcctggctac    240
ctc                                                                243
```

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<210> SEQ ID NO 104
<211> LENGTH: 132
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: miR155 Tat
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<400> SEQUENCE: 104

ctggaggctt gctgaagget gtatgtgtgc cgctttcttc tgccataggg ttttgccac    60
tgactgaccc tatggggaag aagcggacag gacacaaggc ctgttactag cactcacatg    120
gaacaaatgg cc                                                                132
```

What is claimed is:

1. A lentiviral vector comprising an encoded microRNA cluster, wherein the encoded microRNA cluster comprises a sequence comprising (i) at least 90% sequence identity with SEQ ID NO: 1, (ii) at least 90% sequence identity with SEQ ID NO: 2, and (iii) at least 90% sequence identity with SEQ ID NO: 3.

2. The lentiviral vector of claim 1, wherein the encoded microRNA cluster comprises a sequence comprising (i) at least 95% sequence identity with SEQ ID NO: 1, (ii) at least 95% sequence identity with SEQ ID NO: 2, or (iii) at least 95% sequence identity with SEQ ID NO: 3.

3. The lentiviral vector of claim 1, wherein the encoded microRNA cluster comprises a sequence comprising SEQ ID NO: 1, SEQ ID NO: 2, or SEQ ID NO: 3.

4. A lentiviral particle capable of infecting a target cell, the lentiviral particle comprising:

- a. an envelope protein capable of infecting the target cell; and
- b. an encoded microRNA cluster, wherein the encoded microRNA cluster comprises a sequence comprising (i) at least 90% sequence identity with SEQ ID NO: 1, (ii) at least 90% sequence identity with SEQ ID NO: 2, and (iii) at least 90% sequence identity with SEQ ID NO: 3.

5. The lentiviral particle of claim 4, wherein the encoded microRNA cluster comprises a sequence comprising (i) at least 95% sequence identity with SEQ ID NO: 1, (ii) at least 95% sequence identity with SEQ ID NO: 2, or (iii) at least 95% sequence identity with SEQ ID NO: 3.

6. The lentiviral particle of claim 4, wherein the encoded microRNA cluster comprises a sequence comprising SEQ ID NO: 1, SEQ ID NO: 2, or SEQ ID NO: 3.

7. The lentiviral particle of claim 4, wherein the target cell is a CD4⁺ T cell.

8. A modified cell comprising a primary T cell infected with a lentiviral particle, wherein the lentiviral particle comprises:

- a. an envelope protein capable of infecting the target cell; and
- b. an encoded microRNA cluster, wherein the encoded microRNA cluster comprises a sequence comprising (i) at least 90% sequence identity with SEQ ID NO: 1, (ii) at least 90% sequence identity with SEQ ID NO: 2, and (iii) at least 90% sequence identity with SEQ ID NO: 3.

9. The modified cell of claim 8, wherein the encoded microRNA cluster comprises a sequence comprising (i) at least 95% sequence identity with SEQ ID NO: 1, (ii) at least 95% sequence identity with SEQ ID NO: 2, or (iii) at least 95% sequence identity with SEQ ID NO: 3.

10. The modified cell of claim 8, wherein the encoded microRNA cluster comprises a sequence comprising SEQ ID NO: 1, SEQ ID NO: 2, or SEQ ID NO: 3.

11. A method of treating cells infected with HIV, the method comprising:

- a. contacting or having contacted peripheral blood mononuclear cells (PBMC) isolated from a subject infected with HIV with a therapeutically effective amount of an ex vivo stimulatory agent, wherein the contacting is conducted ex vivo;

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b. transducing or having transduced the PBMC ex vivo with a lentiviral particle, wherein the lentiviral particle comprises:

i. an envelope protein capable of infecting the PBMC; and

ii. an encoded microRNA cluster, wherein the encoded microRNA cluster comprises a sequence comprising (i) at least 90% sequence identity with SEQ ID NO: 1, (ii) at least 90% sequence identity with SEQ ID NO: 2, and (iii) at least 90% sequence identity with SEQ ID NO: 3.

12. The method of claim 11, wherein the encoded microRNA cluster comprises a sequence comprising (i) at least 95% sequence identity with SEQ ID NO: 1, (ii) at least 95% sequence identity with SEQ ID NO: 2, or (iii) at least 95% sequence identity with SEQ ID NO: 3.

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13. The method of claim 11, wherein the encoded microRNA cluster comprises a sequence comprising SEQ ID NO: 1, SEQ ID NO: 2, or SEQ ID NO: 3.

14. The method of claim 11, further comprising infusing or having infused the transduced PBMC into a subject.

15. The method of claim 11, further comprising positively selecting or having positively selected HIV-specific CD4+ T cells from the PBMC.

16. The method of claim 11, further comprising immunizing or having immunized the subject with an effective amount of an in vivo stimulatory agent, wherein the immunization occurs prior to contacting the peripheral blood mononuclear cells (PBMC) with the ex vivo stimulatory agent.

17. The method of claim 16, wherein each of the in vivo stimulatory agent and ex vivo stimulatory agent is independently selected from a peptide and a vaccine.

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